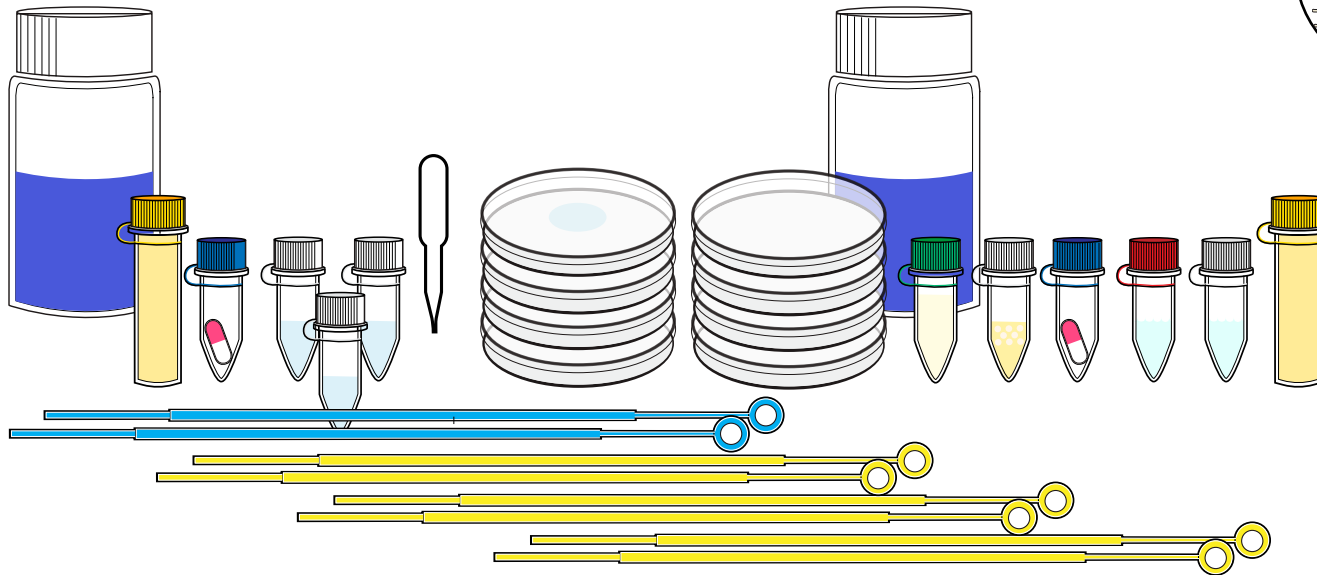


GENETIC ENGINEERING WITH

SMELL-IT KIT™

User Manual



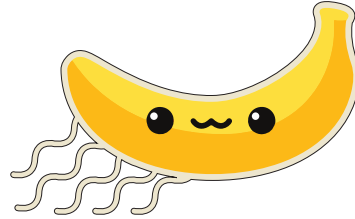
Warning: This experiment includes odors! If you are sensitive to odors or perfumes (e.g., give you headaches, make you nauseous, etc.), the odors in this kit could trigger your sensitivity.

SMELL-IT KIT™

User Manual

Welcome! Let's get started	03	Checkpoint	18
Practicing Safe Science	04	3. Creating competent blank cells	19
How will I learn?	05	Checkpoint	20
Discover your Smell-it Kit	06	4. Transformation	21
What's in the kit?	08	5. Plating cells	22
Storing your kit	10	Checkpoint	23
Technical specifications	11	6. Create LB + substate plates	24
Things you will need	11	7. Streak your cells	26
Implementation timeline	12	8. Smell your results!	27
		Congratulations	28
		Storage, disposal and clean up	29
Your experiment (Experiment protocol)		Glossary	30
1. Creating LB agar plates	14	Troubleshooting	32
Checkpoint	15	More information	33
2. Growing blank cells	16		

Welcome! Let's get started



This User Guide was created to help you get the most out of your Amino Labs Experience. Even if you are familiar with genetic engineering, science or other Amino Labs™ products, please take the necessary time to read through this guide. This will ensure you practice safe science, store, use and get the most out of your Kit and know what to do in case of a spill or accident.

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

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

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

Practicing Safe Science

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

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

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

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

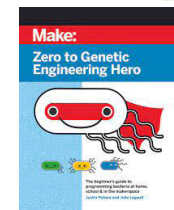
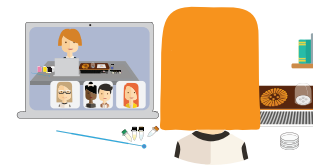
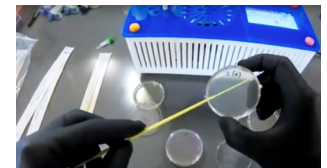
You can download a biosafety poster for your space from www.amino.bio/biosafetyinaction and complete a short safety quiz at www.amino.bio/biosafety-quiz

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

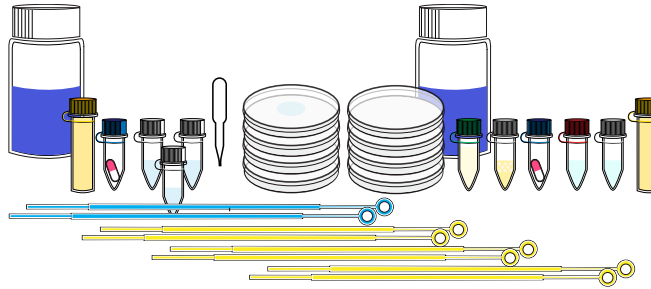
How will I learn?

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

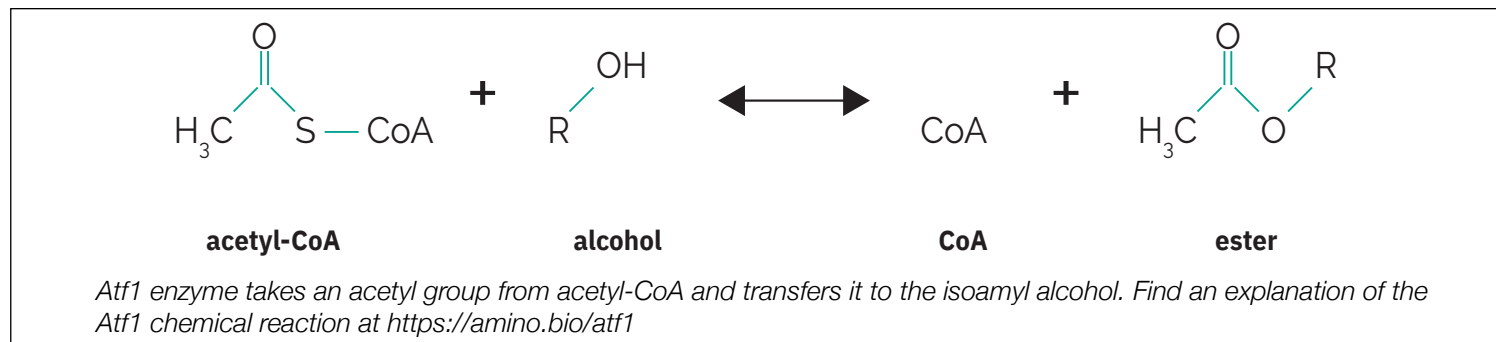
- An essential addition to our ecosystem is the free **Virtual Bioengineer™ simulation** developed with the educators at the Biobuilder Educational Foundation. A 20 minutes guided experience that makes it easy to practice using your DNA Playground™ and Smell-it Kit™ beforehand. It also includes additional information on the manipulations and a more in-depth look into DNA and genetic engineering. We recommend it strongly! Complete it online at www.amino.bio/vbioengineer.
- View **real-time tutorials**, including video tutorials for each day of the genetic engineering experience on our Youtube channel. The genetic engineering days are under 'Engineer-it Kit,' the same procedures as the first 3 days of your Smell-it Kit experience . Subscribe! youtube.com/c/AminoLabs.
- Would you like for an Amino Labs team member to tutor you through your journey? Try the **Virtual Tutoring or Teacher Professional Development sessions**, a multi-day experience via video www.amino.bio/collections/virtual-sessions
- Are you interested in the theory behind the experiment and in going deeper on the science, learning pro-tips and completing more advance genetic engineering?The **Zero to Genetic Engineering Hero book** is for you. Find out more at www.amino.bio/book



Discover your Smell-it Kit™

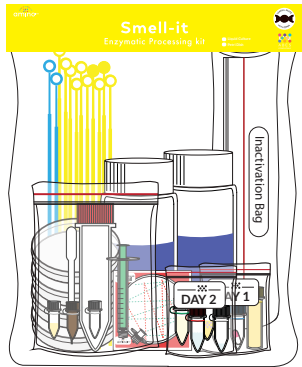


The Smell-it Kit™ has everything needed to insert a DNA Program into bacteria and have them produce what the DNA “tells” them, in this case, an enzyme. This enzyme, called Atf1, converts a musky-smelling substrate, isoamyl alcohol (IA) in your kit into a product, isoamyl acetate, that smells like ‘overripe bananas’.



If you are interested in learning more about enzymes and enzymatic processing, read Chapter 6 of the *Zero to Genetic Engineering Hero* book, www.amino.bio/book.

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, and the different buffers are made to fit perfectly together. Make sure you keep kit components separate!

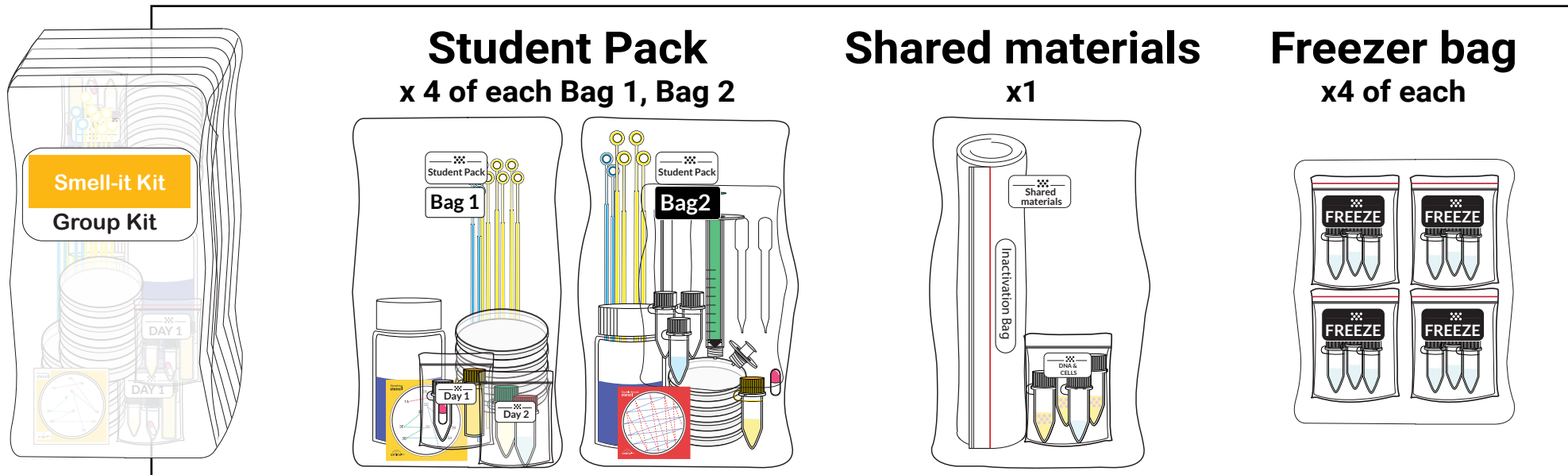


Individual kit size

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

Group kit size

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



Kit Components

Bag 1



Transformation Buffer: A proprietary transformation buffer used in the bacteria transformation procedure to yield high efficiencies. When you adhere strictly to the protocol, this buffer rivals other available competent cells & procedures.¹



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



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



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



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



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



DNA *: A DNA plasmid to program your bacteria.



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



Sterile Water: Sterility is critical when genetic engineering. This Sterile water bottle contains distilled water sterilized in an autoclave to ensure there are no contaminating organisms present. This 50 mL volume, when used with LB agar powder is enough to make 5 LB agar plates.¹



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



Yellow Loops: Large inoculating loops are used for transferring 10 uL of liquid and other tasks. Yellow loops are great for spreading out bacteria after a transformation.



Petri Dish / Plate: 6cm Petri dishes are large enough for typical lab experiments and help save on the cost of reagents as well as reduce waste.



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



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

Bag 2



Agar Powder, Antibiotics for Transformation, Sterile Water, Yellow loops, Petri Dishes: Just like Bag 1, Bag 2 has these necessary items to help you grow cells. 3 of the petri dishes in Bag 2 contain filter paper disks.

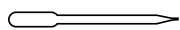


Plate Streaking Stencil: To help you grow the maximum amount of bacteria on your petri dish.

Freezer bag



Isoamyl Alcohol A musky-smelling substrate to be processed by engineered enzyme.



Pipet: Used to transfer the isoamyl alcohol under the paper disks in the petri dishes.

¹ For education purposes only.

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

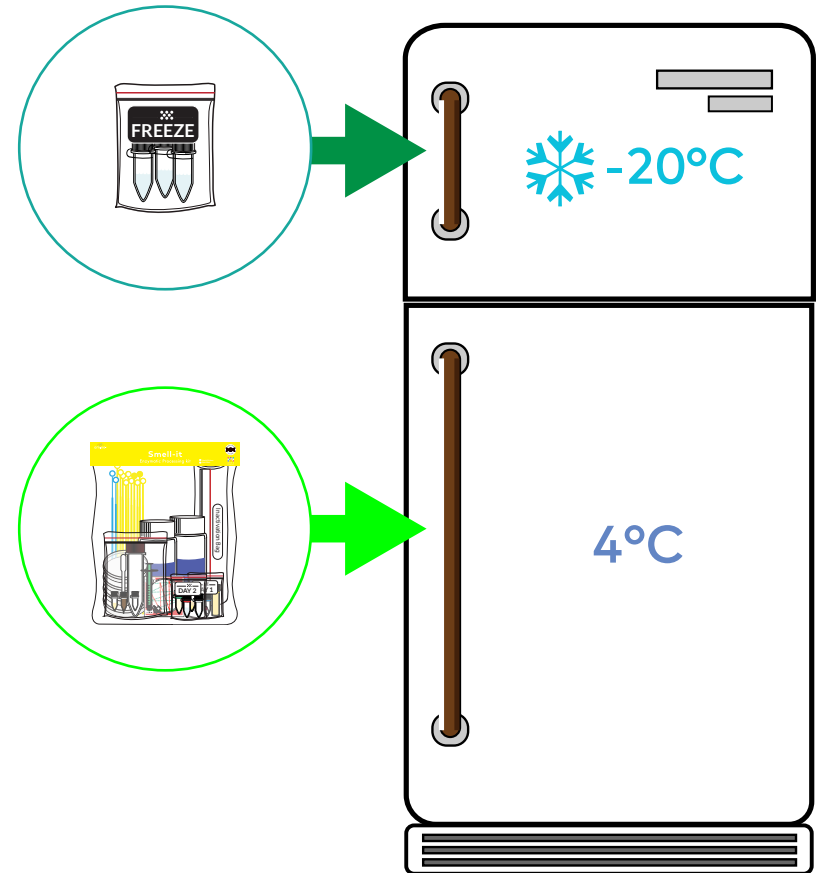
Unpacking and Storing your kit

For a better shelf life and successful experiments:

- place your Kit (Bag(s) 1 & Bag(s) 2) in a standard refrigerator at around 4°C.
- place the smaller Freezer bag in a freezer.

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

Do Not Freeze all of your kit! Only freeze the small bag with *Freeze* on the sticker



Technical Specs

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

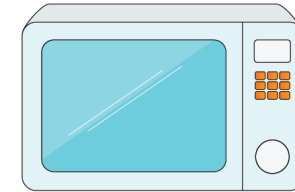
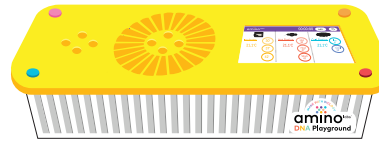
Transformation Buffer: 50 uL tubes
Recovery media: 350 uL tubes
Isoamyl alcohol 3x 30 uL

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

Necessary Equipment

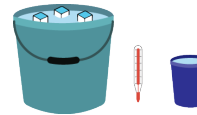
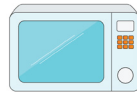
For Best results:

- DNA Playground™ or BioExplorer™
- Microwave



Alternative solution:

- **Microwave**
- **Thermometer** (for 42°C)
- **Timer**



- **Ice bucket or bowl and ice:** This will become your "Cold station" "Ice" for the experiment. Make sure to keep the ice from melting too much during the experiment. You may need a fresh replacement during the experiment if it is warm where you are.
- **Hot water bath or bowl with hot water:** This will become your **Hot station** set to "**Shock/42**" for the experiment. Heat the water to 42°C and try to keep it as stable as possible while you heatshock.
- **Incubator:** This will replace the **Incubator** set to "**37**". If you do not have an incubator (biology or egg one, as long as they set to 37°C), you can create one using an online tutorial Search for DIY incubator on our youtube chanel - Youtube.com/aminolabs - or go to this direct link: <https://www.youtube.com/watch?v=LEsv0Qvbczs>

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

Necessary Safety Supplies



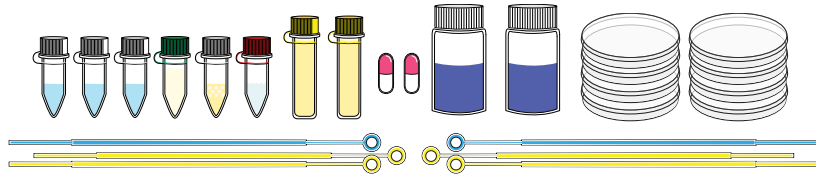
Disposable container 500ml-1L
to hold tubes, loops and other contaminated waste (e.g., yogurt container, plastic cup).

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

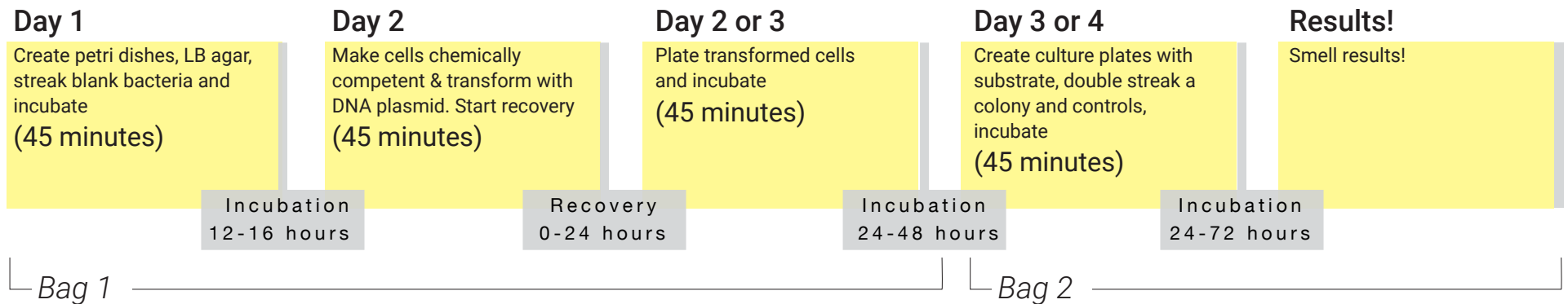
Chlorinated bleach spray
1 regular bottle (or you can mix a 10% solution: 1 part bleach to 9 parts water in a spray bottle)

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

Timeline



Smell-it Kit



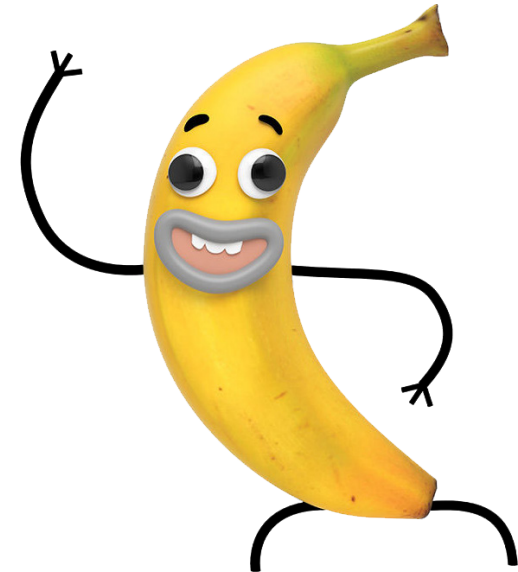
Experiment Protocol

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 are [real-time video tutorials](#) covering the engineering experiment part of your kit. Look for the Engineer-it follow along experiment video!

Find it & more on our youtube channel: [youtube.com/AminobioLab](https://www.youtube.com/AminobioLab)



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

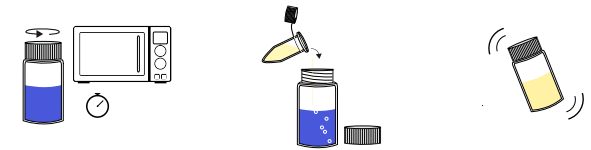
1.1 Label each petri dish with a sharpie-type pen. Label the bottom of the petri dishes (*the side with the star-shaped ring*): **1x** N.S. [your initials] **3x** S. [your initials] of these three, label one **+**, one **-**, and one with an **"e"**



Mix the Agar

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

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



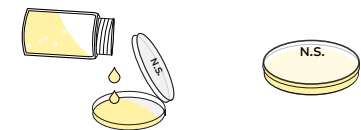
1.4 Add the tube of agar powder to the boiling water. Careful, the water is hot! Some agar powder may "clump" around the lip of the tube due to the water evaporation. This is okay, we have accounted for this possible loss.

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



Make non-selective (N.S.) Plate

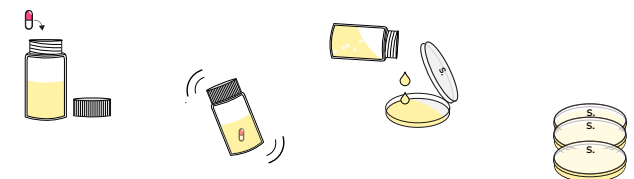
1.6 Pour molten LB agar in the bottom half of your N.S. Petri dish. Enough to fill the petri dish half-full. Swirl the plate to make sure the molten LB agar fills the bottom. If the agar does not cover all the bottom, gently tilt it. Place the lid 3/4 of the way back on so that the agar can cool and dry (solidify).



Make selective (S.) plates

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

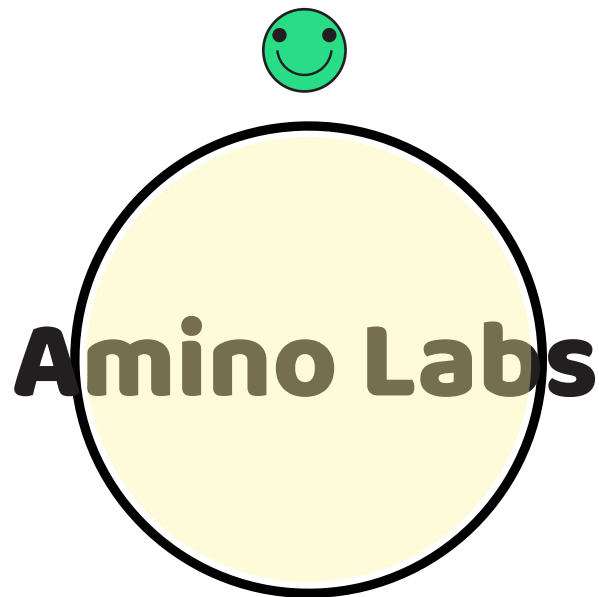
1.7 Once the antibiotic pill is dissolved, pour the molten LB agar into the bottom half of the 3 remaining petri dishes. Place the lids 3/4 of the way back on so that the agar can cool and dry (solidify).



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

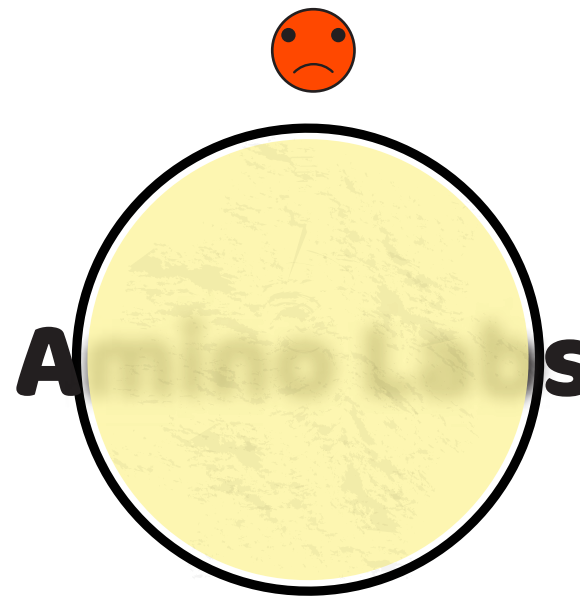
Checkpoint - Agar Plates

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



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

Move on to the next step!



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

Troubleshooting tip

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

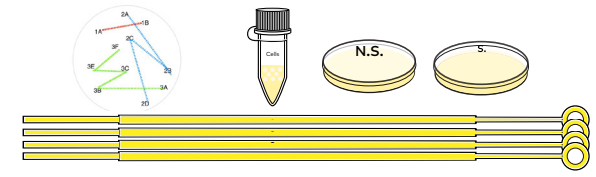
Unfortunately, if the agar does not solidify, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

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

Goal Streak petri dish with provided cells to get fast-growing, well-separated colonies that will be engineered.
Fast growing cells take up DNA the best!

Materials from your kit - BAG 1

Non-selective & "-" selective plate (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. Keep your stencil.

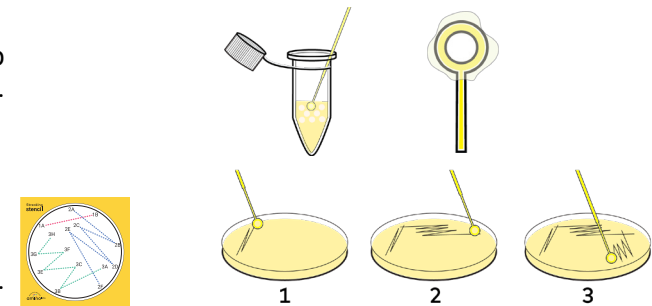
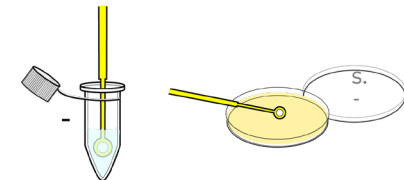


Plate your negative control ("-") plate)

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

2.7 Close your tube of bacteria and place it back in the fridge in a zip-lock bag if you want to keep them, discard them in the Inactivation bag if you do not. You no longer need them for this experiment.



Incubate Overnight

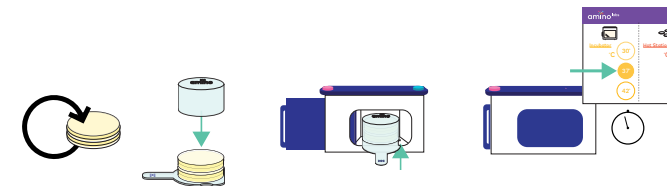
2.8 Flip your streaked N.S. & S. "-" plates upside down and stack them in a pile in an incubator set to 37°C. If you are using the DNA Playground, put your stack of plates on top of the incubator paddle, set the incubator humidity chamber on top and slide in the incubator. Close the incubator door and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

If you are using The DNA Playground or a commercially-made incubator at 37°C:

It is important to do the next steps in 12 to 24 hours so that your cells will be in their optimal growth phase.

If you are using a homemade incubator:

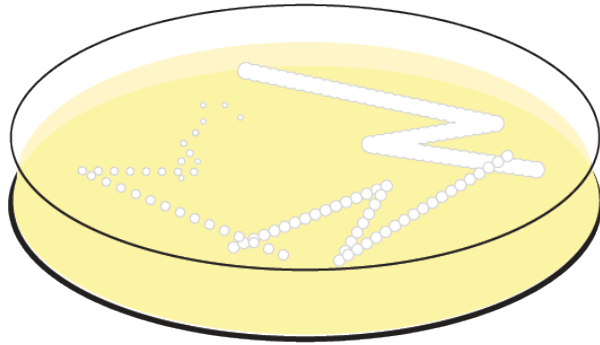
It must incubate between 35°C and 37°C. If the cells are below 37°C, it will take longer for them to incubate - you may have to wait up to 48 hrs until you see small colonies on your plate.



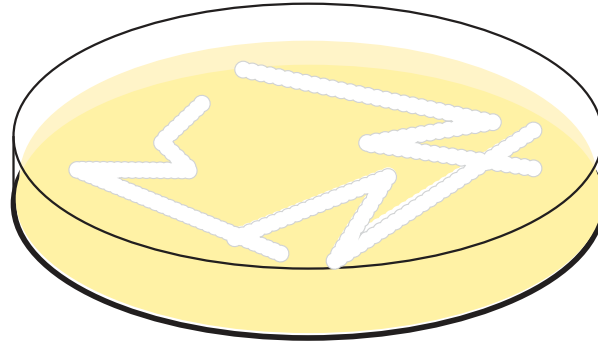
Teacher Tip! If you are in a classroom setting and your class is 24 hours-30 hours apart or more, you can initially grow your cells at 30 C overnight and then increase the temperature to 37 the day of making competent cells.

Checkpoint - Non-selective plate & blank cells

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

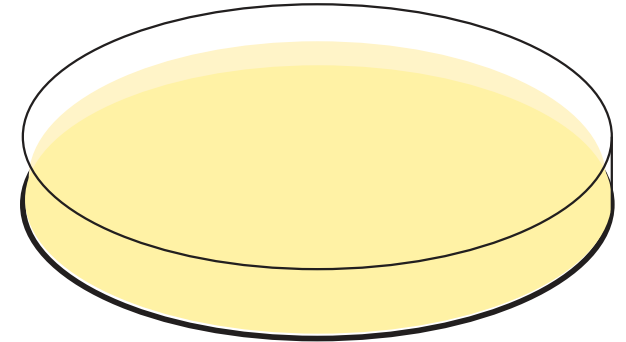


A perfect N.S. plate has lots of small individual white colonies (dots) of fast-growing bacteria after incubation. Proceed to the next page.



An okay N.S. plate has bacteria growth but few or no individual colonies (dots) after incubation. Continue with the experiment by selecting the colonies that are on the edges of the dense lines of bacteria.

Note that your results may not be as good as when you start with a perfect plate. Don't worry though, practice makes perfect, and our Success Guarantee means you will have the chance to be successful if this try doesn't work.

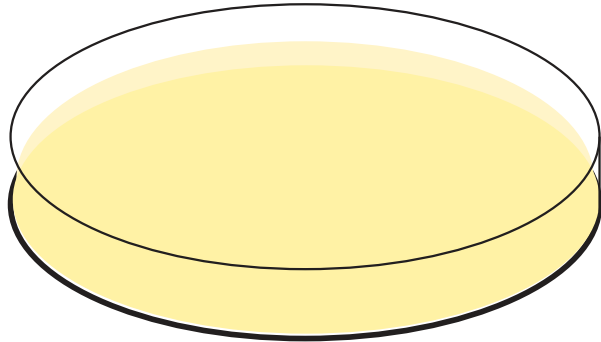


If you see no growth on your N.S. plate:

1. If your incubator was not at 37°C or is homemade, incubate for another 24 hrs.
2. If you are certain you incubated at 37°C, or incubated for 48 hrs and still have no colonies, you might not have had cells on your loop when you streaked. Repeat Step 2: Growing Blank cells on this plate.
3. If you still have no colonies after repeating Step 2, complete the guide at www.amino.bio/troubleshoot

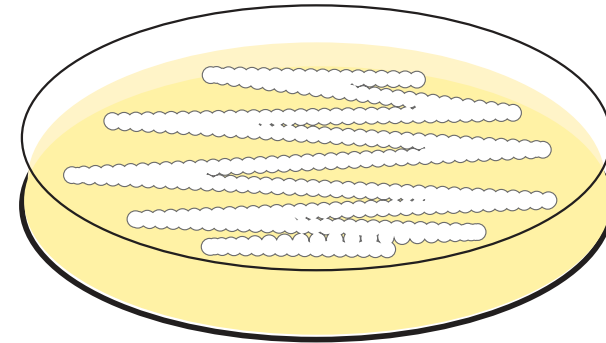
Checkpoint - Selective plate and “-” control

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



A perfect “-” plate has no growth on it. This is because the antibiotics do not allow the blank cells to grow. This means that you made your selective plates properly.

Move on to the next step.



If you see growth on your “-” plate, this suggests that there is not enough antibiotics.

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

3. Making Chemically Competent Cells Day 2, 10-15 minutes

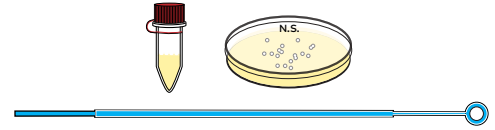
Goal Pick small colonies, and suspend the bacteria in cold transformation buffer, enabling bacteria to better take up DNA.

Materials from your kit

(1) Streaked N.S. Plate

(1) Blue Loop

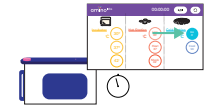
(1) T. Buffer Tube



Prepare

3.1 Make sure you have completed the Non-Selective Plate & Blank cell + Selective Plate and "-" control Checkpoints. You can discard the S. "-" plate in your inactivation bag.

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

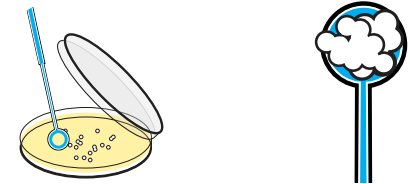


Mix the cells and T. Buffer

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

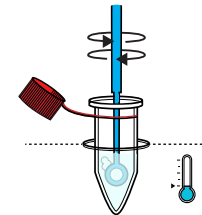
3.4 Take a blue inoculating loop and gently scrape it over small, well-separated colonies on your N.S. plate. Colonies that are ~1mm in diameter work the best. You want to collect ~10 or 20 of these colonies on your loop, enough so that you can see that the center of the loop is full.

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.

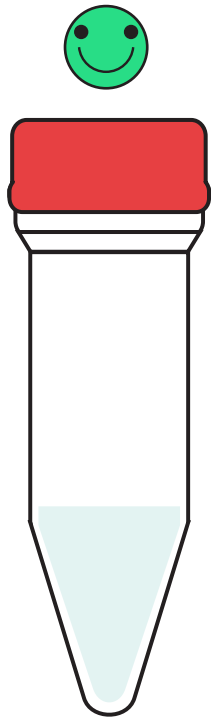


When successful, the solution should be slightly cloudy, and there should be no clumps floating in the solution. You may have to mix vigorously for up to 45 seconds. If you see clumps, keep blending. You can lift the tube out of the cold station to see whether the T. Buffer is cloudy and the clumps are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

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

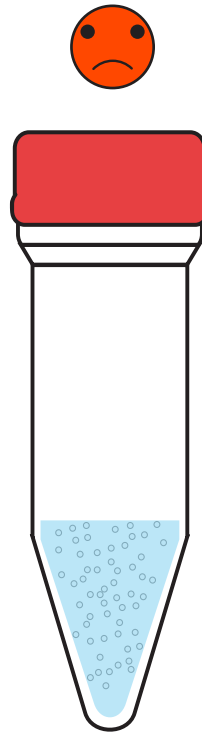
Checkpoint - Competent cells

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

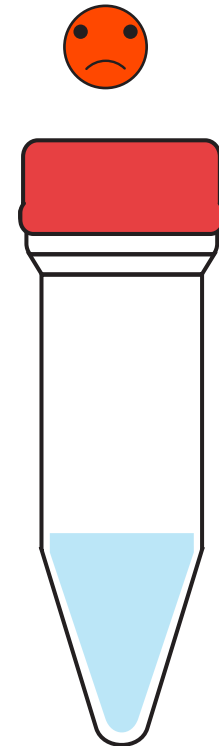


Cloudy liquid - Perfect!
The cells can now take up DNA.

Move on to the next step.



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



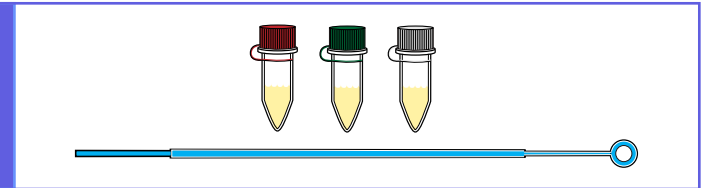
Clear liquid? Add more cells and mix until the liquid is cloudy like the image on the left.

4. Transformation Day 2, 45-60 minutes

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

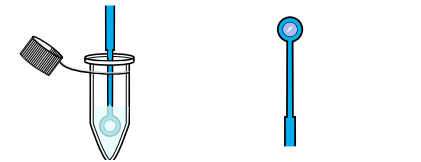
Materials from your kit - BAG 1

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



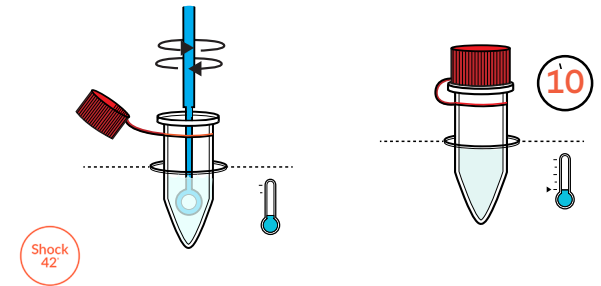
Take the DNA

4.1 Tap your DNA tube on the table or briefly microcentrifuge to make sure most of the DNA is at the bottom. Dip a Blue inoculating loop into the DNA tube and twist a few times. When you pull the loop out of the DNA tube, the hole of the loop should have liquid in it. Each tiny droplet of the liquid contains thousands of DNA plasmids, small circles of DNA programs that you will use to engineer your cells. Make sure you can see liquid in the loop!



Mix the competent cells & the DNA

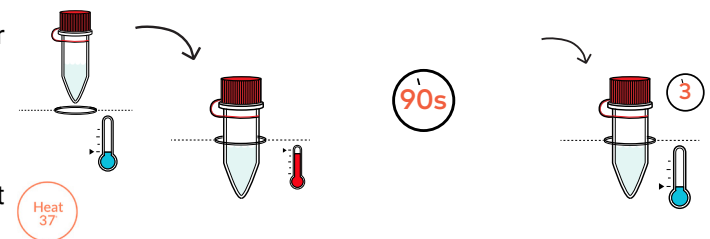
4.2 Slowly dip and spin the inoculating loop containing DNA into the competent cells you made in the previous step. Stir/swirl for 5 seconds to fully mix. Do this while the tube is on Ice / Cold Station. Discard the loop. * Do not reuse the inoculating loop! *



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

HeatShock

4.4 It is now time to get the DNA into the cells. Heat shock your cells, T. Buffer, and DNA solution by moving your tube to the Hot Station (42°C) / Water Bath (42°C) for 90 seconds

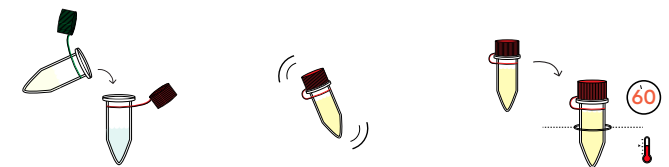


4.5 After 90 seconds, immediately place your tube back on Ice / Cold Station for 2 minutes.

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

Recovery

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



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

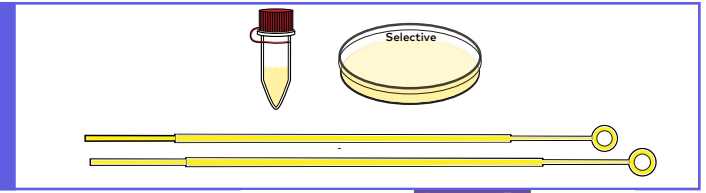
After the initial 30 minutes of recovery, you can wait up to 24 hours before moving onto the next step.

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

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

Materials from your kit - **BAG 1**

- | | |
|-----------------------------------|---|
| (1) Selective Plate {from step 1} | (1) Transformed Cells {from prior step} |
| (2) Yellow Loop | (1) "+ Cell" Positive Control tube |



Prepare

5.1 Turn on your Incubator at 37°C.



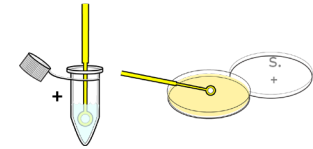
Plate your transformed cells ("e" plate)

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 keep them just in case you do not get colonies since you can always pour the remaining recovered cells onto the selective plate and incubate them after your step 6. incubation.



Plate your positive control ("+" plate)

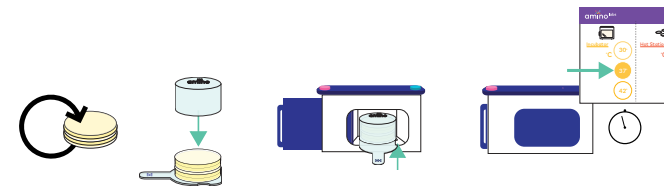
5.3 Get the stab of "+ cells" and your Selective LB Agar plate labeled "+". Using a single yellow loop, dip into the + cells stab and spread them across your agar plate in any pattern of your choosing.



Incubate

5.4 Once there is no liquid pooling on any of the plates, place the plates upside down in the Incubator. If you are using a Minilab incubator, make sure to use the humidity chamber as well. Inverting the plates ensures that water vapor in the plates keeps the LB agar surface moist.

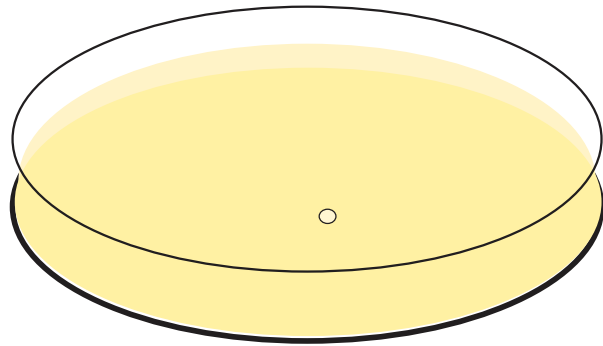
5.5 Incubate for 24-48 hours until you see one or more colonies.



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

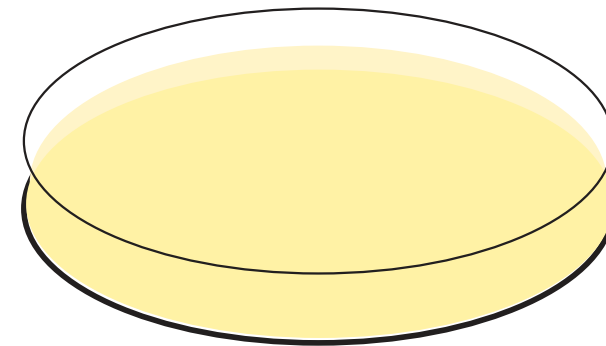
Checkpoint - Did your engineered cells grow?

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



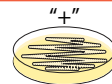
A perfect “e” plate has one or more colonies on it. Remember, even a single colony is a success. One colony is all you need to move onto the next step. Congratulations!

Move on to the next step.



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

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



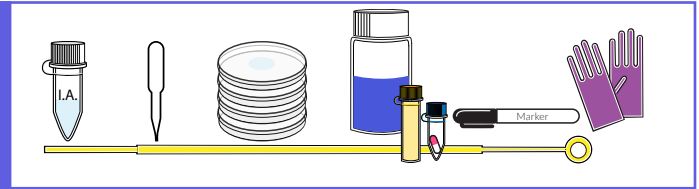
You can continue your experiment with a colony from your “+” plate if the + cells grew. Try to select a single, isolated colony if you can.

6. Create LB Agar Plates for the Enzymatic Reaction 25 min.

Goal Create selective LB agar plates with the necessary substrate for an enzymatic reaction to take place.

Materials from your kit - BAG 2

- | | | |
|-------------------------|--|--------------------|
| (1) 50 mL sterile water | (4) 6 cm petri dishes with paper disks | (1) Sharpie marker |
| (1) LB agar powder | (1) Pipet | |
| (1) antibiotic pill | (1) Yellow loop | |



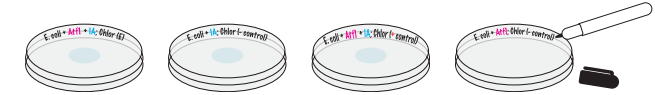
Prepare

6.1 Using a sharpie-type pen, label the bottom of the petri dishes like so : (the bottom is the side with little tabs)

For the 3 plates with the paper disks:

- 1x E. coli + Atf1 + IA; Chlor (E);
- 1x E. coli + Atf1 + IA; Chlor (+ control);
- 1x E. coli + IA; Chlor (- control);

For the 1 plate without a paper disk: 1x E. coli + Atf1; Chlor (- control);



A note on your plates:

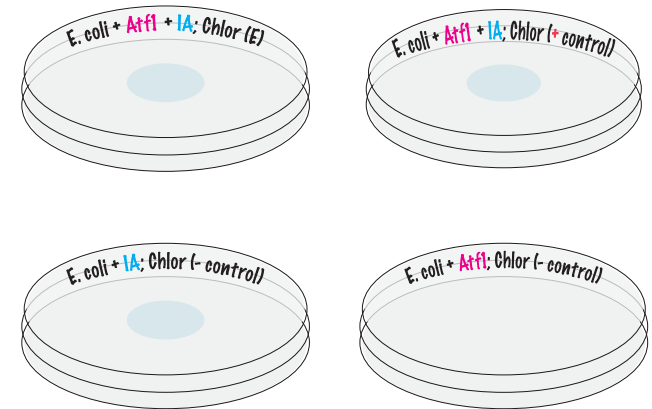
E. coli + Atf1 + IA; Chlor (E): Your experimental sample. You should smell overripe banana on this plate. The organism is E. coli expressing Atf1, the protein enzyme you engineered. The plate will contain the substrate isoamyl alcohol, which we may refer to as 'IA' from now on.

E. coli + IA; Chlor (- control): Your negative control. The organism you will streak is non-engineered E. coli cells - they do not express Atf1. The plate contains the IA substrate. These cells will grow on the selective LB agar plates, but since they do not express the Atf1 protein enzyme, the IA substrate will not be processed. Therefore the plate should smell similar to the original IA substrate in the tube.

E. coli + Atf1 + IA; Chlor (+ control): Your positive control is for you to streak with positive (+) cells included in the kit that express Atf1. You will add IA to the plate. You should smell overripe banana on this plate.

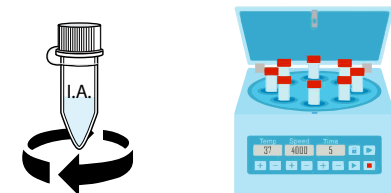
E. coli + Atf1; Chlor (- control): Your second negative control. In this instance, you will streak your plate with some Atf1 expressing engineered E. coli (+ cells). However, you do not add IA substrate. This control will verify that the E. coli themselves do not produce the overripe banana smell.

All your experiment plates will be selective.



Prepare the IA substrate

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



Make selective LB Agar

6.3 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.

6.4 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!

6.5 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.

6.6 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.

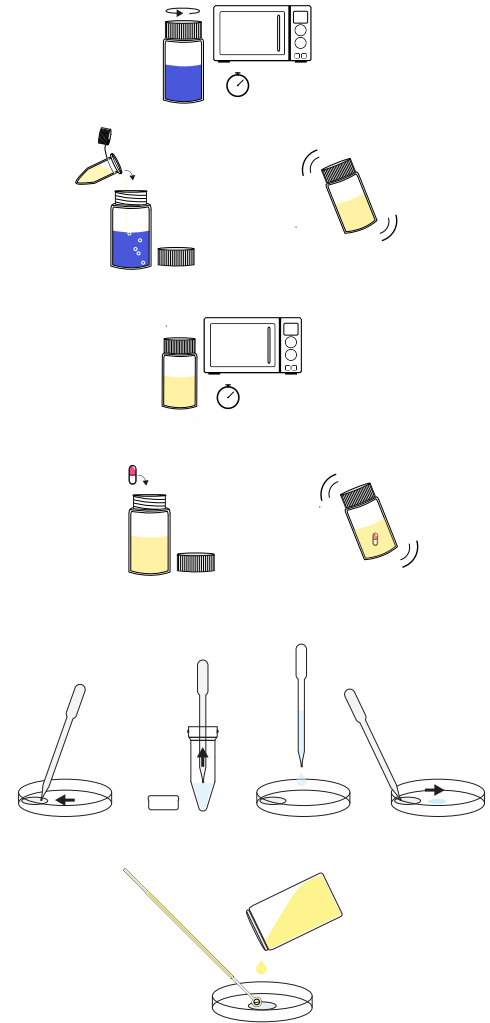
6.7 Add the antibiotic pill to the bottle of agar and gently swirl for a few minutes until the contents of the pill have dissolved. Do not introduce bubbles into the LB agar: don't swirl too vigorously. The gelatin capsule may not fully dissolve. The important thing is that the contents of the capsule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

Once the antibiotic pill is dissolved, and while your LB agar is cooling, you will add the IA substrate to the paper disks in the 3 plates labeled with 'IA': *E. coli* + Atf1 + IA; *Chlor* (E); *E. coli* + Atf1 + IA; *Chlor* (+ control); *E. coli* + IA; *Chlor* (- control) . You must do this next step rather quickly before your LB agar starts solidifying.

6.8 Using the pipet included in your kit, push the paper disks to the side of the three petri dishes. Then, using the pipet, transfer an entire tube of IA substrate to the middle of one of the plates, and quickly use the pipet to push the paper disk over the top of the liquid to trap it in the paper disk. Do the same action with the other 2 plates, using a new tube of IA substrate for each.

6.9 Pour your Selective LB agar into each plate. For the 3 plates with paper disks, use a sterile yellow loop to hold the paper disk at the bottom of the plate when you pour in the LB agar - the disks may begin to float, but the loop will help you keep them at the bottom of the plate. Use the same yellow loop for all plates.

6.10 Place the lids back on. Allow your dishes to cool and solidify. All the plates are used in the next step.



7. Streak your cells 15-25 minutes + 24-48 hours wait time

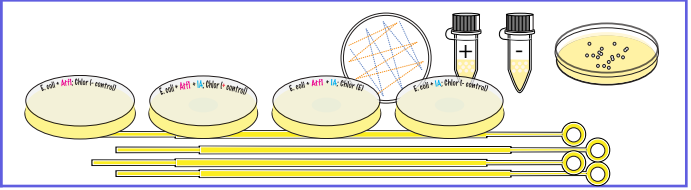
Goal Grow cells on plates with the IA substrate so that an enzymatic reaction can occur.

Materials from your kit - BAG 2

Agar plates from previous step
Your engineered cells

(1) Positive control cells
(1) Negative control cells

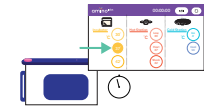
(4) Yellow Loops
(1) Double-streak stencil



Prepare

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

37°



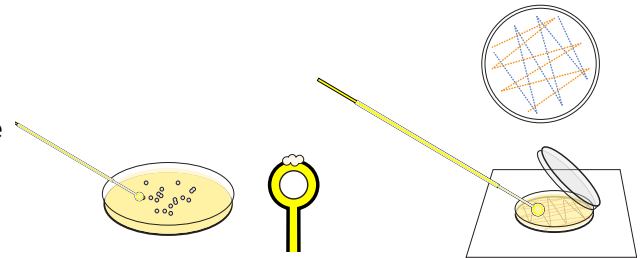
7.2 Streak the E. coli + Atf1 + IA; Chlor (E) plate with your engineered cells.

7.2 Place your E. coli + Atf1 + IA; Chlor (E) plate on top of the double zig zag pattern stencil.

7.3 Using a new yellow loop, pick* one or more colonies from your engineered cells plate (from step 5) and trace the two zigzags on the E. coli + Atf1 + IA; Chlor (E) plate.

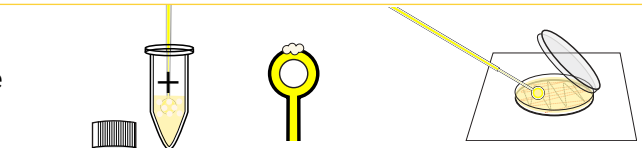
*You pick a colony by touching the end of the loop to it, gently rubbing it.

7.4 Discard your loop.



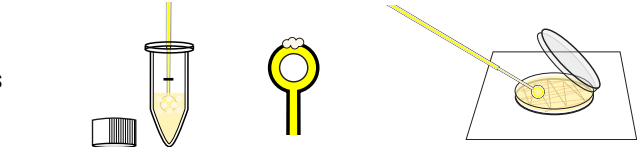
7.5 Streak the E. coli + Atf1 + IA; Chlor (+ control) plate with your positive control cells.

7.5 Use a new loop to repeat steps 7.2 - 7.4 with the E. coli + Atf1 + IA; Chlor (+ control) petri dish and the positive cells stab. Instead of picking a colony, in this case, you will dip the yellow loop in the stab of cells.



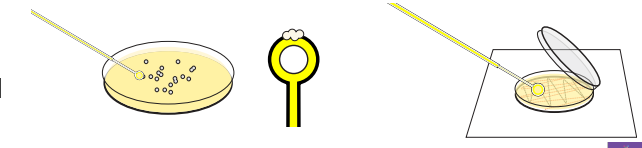
7.6 Streak the E. coli + IA; Chlor (- control) plate with your negative control cells.

7.6 Use a new loop to repeat steps 7.2 - 7.4 with the E. coli + IA; Chlor (- control) petri dish and the negative cells stab. Instead of picking a colony, in this case, you will dip the yellow loop in the stab of cells.



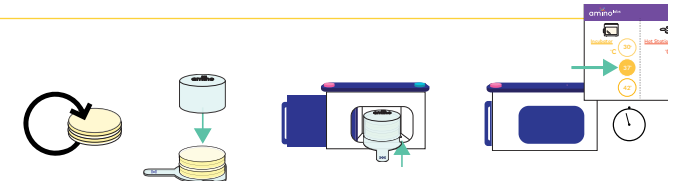
7.7 Streak the E. coli + Atf1; Chlor (- control) plate with your positive control cells.

7.7 Use a new loop to repeat steps 7.2 - 7.4 with the E. coli + Atf1; Chlor (- control) petri dish and your engineered cells plate.



Incubate Overnight

7.8 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. If you have an Amino Labs minilab, remember to close the incubator door and lock it!

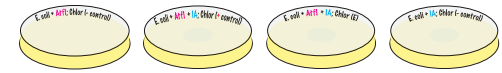


8. Smell your results! 15 minutes

Goal Smell that banana smell! Verify if your enzymatic reaction took place.

Materials from your kit

Your incubated experiment plates



Smelling reagents safety pro-tip:

When smelling materials in any lab or engineering environment, you should never place your nose directly over the top of the container opening and inhale. This is very dangerous because the material you want to smell could be toxic or overwhelming. Wafting is a safe technique to use when smelling reagents.

Visit: <https://amino.bio/wafting>

8.1 Opening your plates to smell your results

During incubation, as the IA substrate diffuses through the agar to the surface, the engineered cells absorb it, and the engineered enzymes being expressed process the IA (isoamyl alcohol) into a different molecule called isoamyl acetate. This molecule has the smell of overripe bananas!

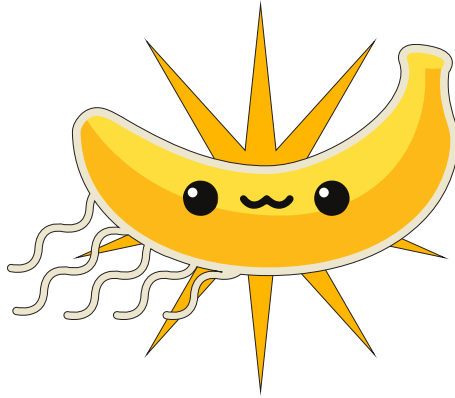
Both your (E) plate and your (+ control) plate should smell like overripe bananas. As you open the lids of the plates, the smell will disappear into the ambient air and you will need to wait for it to replenish. Keep incubating them for the smell to replenish.

Keep in mind that smells are subjective - while most will agree that the isoamyl acetate smells like overripe banana, you may not. The important thing is to notice that the smell is different from a non-processed IA (isoamyl alcohol). To notice the difference in smells, have a whiff at the (- control) plate with IA (*E. coli* + IA; *Chlor* (- control)) and an empty tube of IA.

Note

If you cannot see any growing cells after 48 hours, or if your (E) plate does not smell the same as the (+ control) plate, your experiment may have failed. Don't be discouraged. In science especially, failure is a chance to learn more. Complete the troubleshooting guide at www.amino.bio/troubleshoot and claim your free kit to try again, as part of our success guarantee.

Congratulations!



You did it! Using your Smell-it Kit™, you inserted a DNA Program inside single-celled organisms called bacteria and had the bacteria execute that DNA Program to produce something for you, just like scientist and industries do every day inside their large laboratories. We hope you enjoyed the experience and will continue to experiment in the field of genetic engineering.

Show off your results to your friends, our community and us too! We are 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, 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](https://www.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 **Deoxyribo**nucleic 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, like 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°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 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

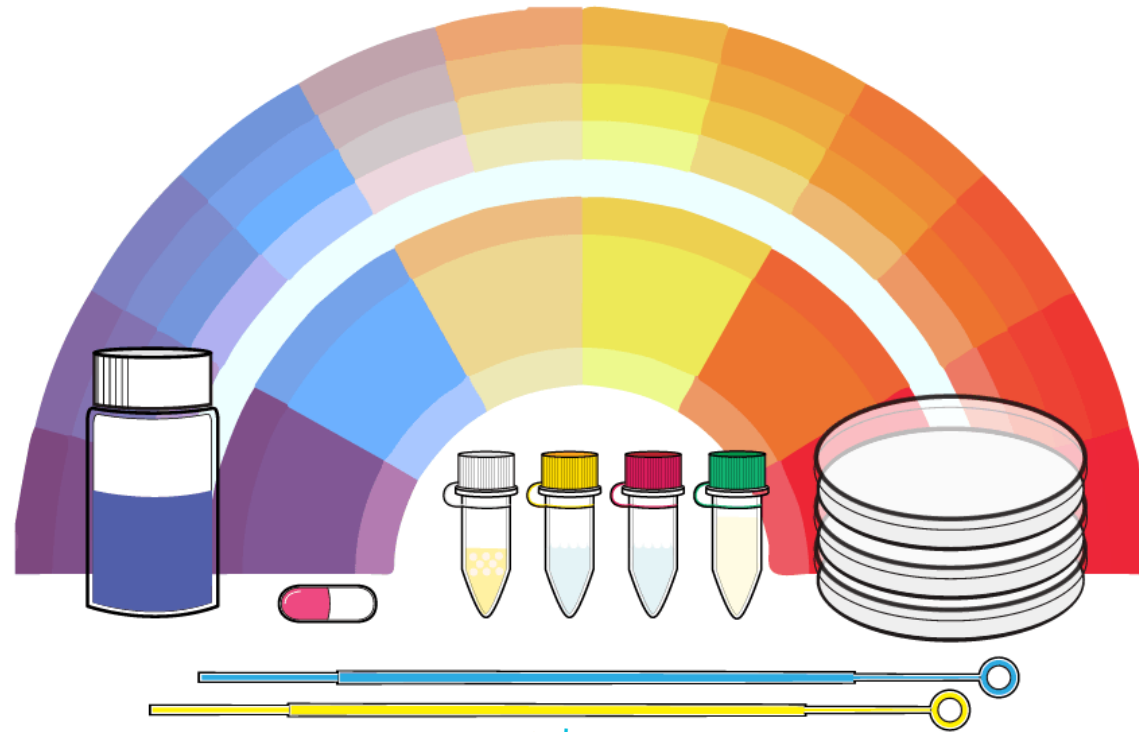


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