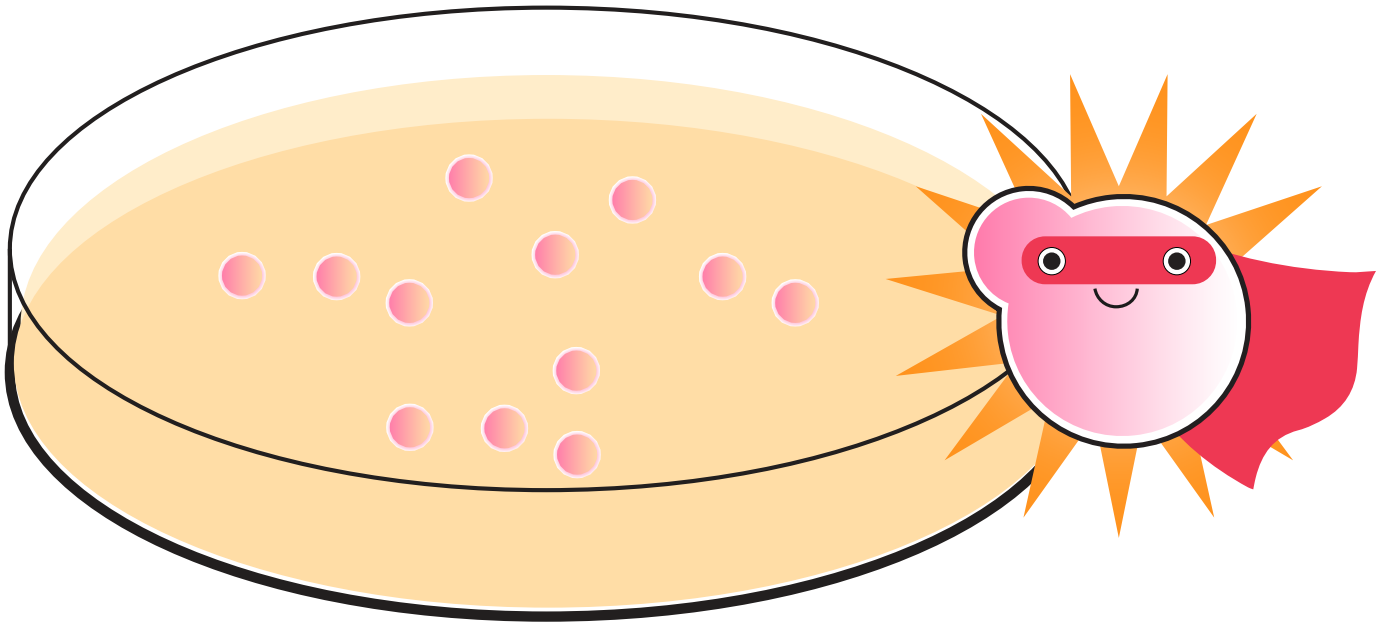
YEAST

ENGINEER-IT KIT™

METABOLIC SELECTION - DNA PINK

INSTRUCTION MANUAL



Version 1.2, Oct 2023

ENGINEER-IT KIT™

INSTRUCTION MANUAL

Getting Ready

The Experiment

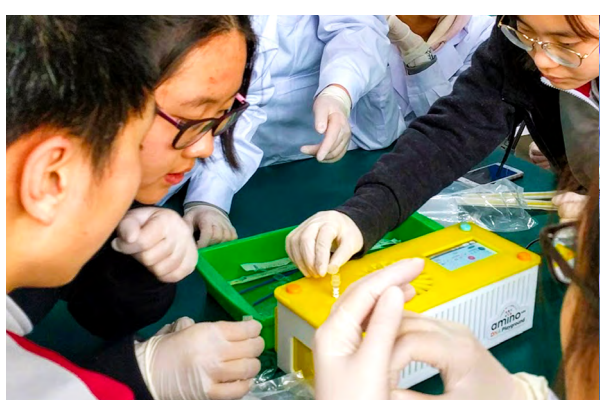
More information

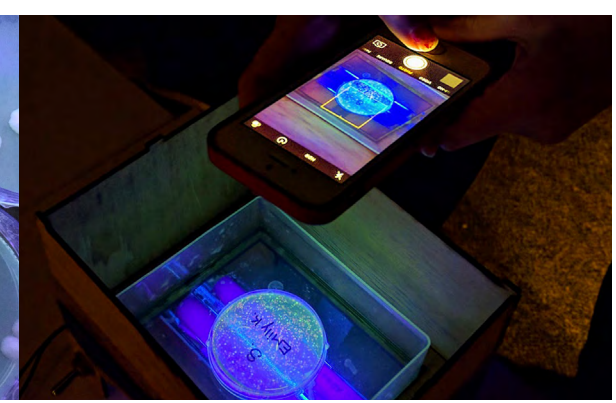
Welcome! Let's get started

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 as it describes 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" and describes how to keep your creations, store or dispose of any leftover ingredients and general clean up instructions. The final section is there to help you and includes a glossary, troubleshooting, and our contact information.

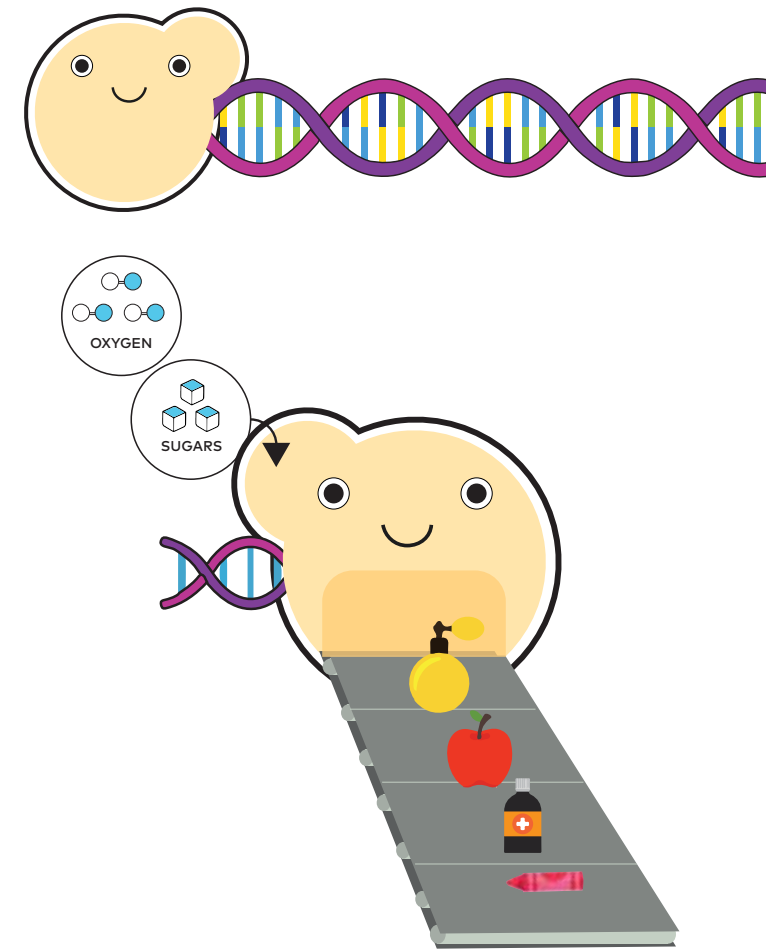
Amino Labs is excited to welcome you to the world of the genetic engineering with the YEAST Engineer-it Kit™, and our entire ecosystem of easy-to-use, easy-to-succeed at products! **Following this guide will help ensure that you are getting the most out of your current and future experiences to keep on making new creations with DNA. Have fun!**





Genetic engineering with yeast

Yeasts have been used for century to make and ferment delicious food: breads, cheeses, yogurts, soy sauce, root beers, alcohol and more. Today, yeast are part of a revolution in sustainable advancements across food, energy, health, and materials thanks to genetic engineers. Genetic engineers (or biological engineers) produce medicine, food, fuel, household products, and new materials by using yeast to “read DNA” and create from these blueprints.



If you’ve learned about bacterial genetic engineering, you’ll find that yeast engineering is similar. Yeast are also reminiscent of miniature factories, and they can follow DNA programming that has been inserted in them through genetic engineering and create products in response. Each yeast produces a small quantity of the product which, when cultivated in large vessels, generate significant amounts of flavours, scents, medicine, plastic compounds, and more. These can then be extracted and used by industries and individuals. Since yeast multiply rapidly when they are fed the right sugars and amino acids, and are kept in a controlled environment, creating products through genetic engineering can be sustainable and safe.

Thanks to the hard work of scientists around the world, programming organisms is improving our quality of life and keeping diseases at bay. Already in the hands of hundreds of millions of people each day, the number of genetically engineered products will continue to rise in the future. And now, you too can program organisms! Amino Labs’ kits have everything you need to engineer organisms whether for the first time or the hundredth!

Practicing safe science

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

The kit in your hands contains only non-pathogenic ingredients. These are part of the biosafety Risk Group 1 (RG1), also called Biosafety Level 1 (BSL-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 microorganisms 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 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 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.

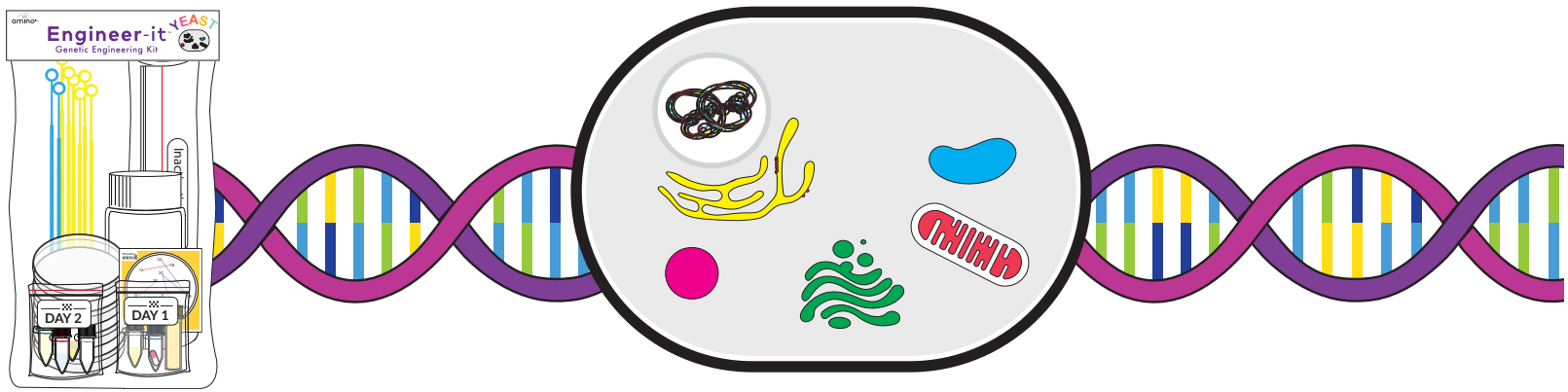
We also have additional resources to help you go further:

An essential addition to our ecosystem are the free **Virtual Bioengineer™ simulations** developed with the educators at the Biobuilder Educational Foundation. These simulations are 20 minutes guided experiences that make it easy to practice using a DNA Playground™ and experiment kits beforehand. While the simulations focus on bacteria experiments, you can still use them to learn about these similar experiments as they include additional information on the manipulations and a more in-depth look into the kit components. Complete online at www.amino.bio/vbioengineer. A YEAST Edition of the virtual bioengineer simulators is coming in 2022.

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

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

Discover the YEAST Engineer-it Kit™



The Yeast Engineer-it Kit™ has everything you need to insert a DNA plasmid (we sometimes call this a DNA program) into yeast and have them produce what the DNA "tells" them. Note that the yeast, the agar, and all the different buffers were made to fit perfectly together, so make sure you keep kit components separate if you have more than one kit.

Speaking of DNA programs, the DNA program in your kit encodes new functionality that changes the yeast. For example, in this yeast kit, once engineered, your yeast will produce a colored protein.

The Yeast Engineer-it Kit™ in individual size helps you grow and engineer yeast once using the DNA program provided. This can be done by an individual or a small group. You'll notice you get four petri dishes. That is because you will also complete experimental "control" samples. Control petri dishes allow scientists and scientific explorers to see if their experiments succeeded, failed, or both! These instructions will show you how to do a positive control and a negative control and explain how controls work.

The Yeast Engineer-it Kit™ in Group size contains eight student bags, each of which is suitable for one person or team to grow and engineer yeast with DNA. This kit can be used alone or in a small group (with parent supervision if you are 16 or under). You will find kit descriptions of the kit's content in the next pages.

What does your yeast kit allow you to do?

Yeast are single-celled microorganisms. They are part of the fungi kingdom. Individual yeasts can only be seen with a microscope, but they reproduce and form colonies that we can see with the naked eye. Yeasts reproduce when one cell splits into two cells through a process called budding. This occurs in about 90 minutes. Under perfect conditions, a single yeast could grow into over one hundred thousand yeast in one day!

As we saw in earlier pages, yeast are tiny living units that function like mini-factories. Each yeast is told how to use its factory-like capabilities by its DNA. DNA is like a computer program; it is the set of instructions that tell the cell how to function.

In this Yeast Engineer-it Kit, you get a DNA plasmid (sometimes referred to as a DNA Program). DNA plasmids are also a set of instructions for the yeast but much smaller than its internal genome DNA. A DNA plasmid has only a few functions as compared to the yeast genomic DNA that encodes thousands of functions.

It is by adding new plasmids in the blank yeast provided through the process of genetic engineering that we can get them to produce things for us. For example, in this kit, you will engineer yeast with DNA plasmids containing the instructions on how to produce a colorful pigment.

Since DNA is a very hydrophilic (water-loving) molecule, it won't normally pass through the yeast's cell membrane. To make yeast take in the DNA plasmid, the yeast must first be made "competent." This means creating small holes in the yeast cells by suspending them in a solution with a high concentration of lithium (the transformation buffer). DNA can then be forced into the cells by incubating them with the DNA and performing temperature changes from warm to hot and then back to the ideal growth temperature. This process that causes the yeast to take in DNA and begin reading the DNA instructions is called a "transformation".

In your kit, the yeast you will find is a standard lab strain (W303A) commonly used in research. This yeast is safe for use in your home, the classroom, a community, or maker space anywhere in North America.

To engineer the blank yeast, you will need to grow them on nutrient agar petri dishes (also called plates) before you insert the DNA plasmid. Nutrient agar is a Jell-O-like staple food source for the yeast, which you will pour into petri dishes in the first step of your experiment. After engineering your yeast, you will again grow them on agar petri dishes. Remember, agar petri dishes are both the food source for microorganisms and the substrate they grow on.

So, when making your agar petri dishes, you will create two types of agar: non-selective and selective. The non-selective agar allows any yeast to grow, including your blank yeast. Selective agar only allows specific yeasts to grow, including the one yeast you will engineer. **If you have completed other Amino Labs' bacteria kits or the Yeast Engineer-it Kit with Artic Green DNA, you'll notice a difference when making your petri dishes. In this kit, you will first make and pour selective agar in three selective petri dishes. Then, you will add a powder to the remainder of the agar in the bottle, which will turn it into non-selective agar so that you can pour your N.S. petri dish! You create the petri dishes in this order because this kit uses metabolic selection, as opposed to the antibiotic selection that other Amino Labs kits use.**

What is Metabolic selection?

When creating the petri dishes for this experiment, you will be creating the selective and non-selective plates in inverse order compared to other Amino Labs kits. You will start by making your three selective petri dishes using S.C. agar (or SC-Ura agar). S.C. agar contains all the essential nutrients except a molecular called URA (uracil). Only your engineered bacteria can make their own uracil and thus will grow on these plates. Your blank cells cannot grow on these selective plates that lack uracil!

The DNA plasmid you will use in this kit contains the instruction (gene) to enable yeast to synthesize (create) uracil from the other molecules present in the S.C. agar. The gene is called URA3. This means that with this gene, the engineered yeast can create the uracil they need to survive and grow by using other molecules present in the media. Cool! Therefore, the SC-Ura agar 'selects' for yeast that have the gene for uracil production, making sure you are only growing your engineered yeast on your selective petri dishes.

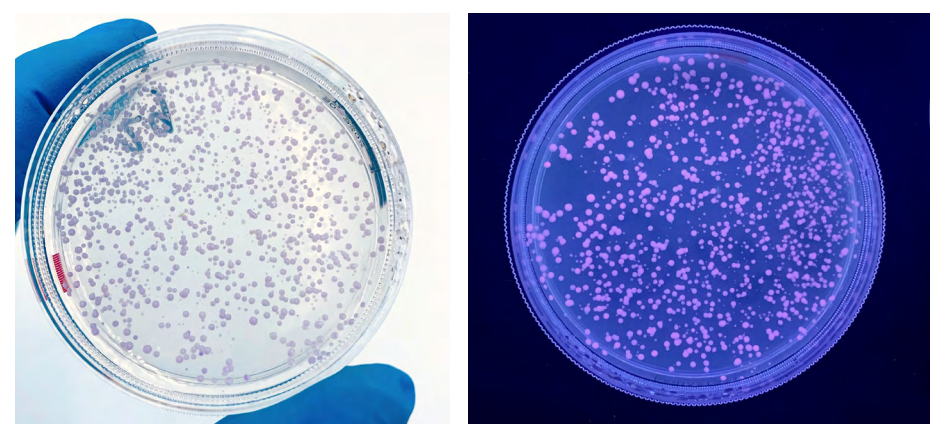
To make your Non-Selective petri dish (the petri dish that allows you to grow blank yeast), you will simply add a nutrient powder from the “N.S. nutrient” tube to the leftover SC-Ura media in the bottle. This will change the SC-Ura media from a minimal media to a rich media because the N.S. Nutrient contains uracil. Once you add the nutrient powder to your SC-Ura media, it stops being selective since it now contains all the nutrient blank yeast need. So, even though the blank cells cannot create their own uracil, you’ve now added lots of it to the agar that the blank cells can now use to grow and thrive.

This type of selection is called metabolic selection. Other Amino Labs kits use what is called antibiotic selection. Antibiotic selection is the process of adding antibiotics to media that already contains all the nutrients the bacteria or yeast need to grow in order to make the media selective. You then engineer the bacteria or yeast with DNA that contains the instruction (gene) to be resistant to that antibiotic. (Don’t worry, this does not create troublesome antibiotic-resistant microbes, and you can learn more about this here: https://amino.bio/blogs/news/engineer-it-kit-safety)). With antibiotic selection, you have to add something (the antibiotic) to the media to make it selective, so you start by pouring your non-selective plate before adding the antibiotic.

Once you have made media and poured selective and non-selective petri dishes, you will use a DNA plasmid (DNA program) to engineer your blank yeast into yeast that produces its own uracil and a color pigment!

The color pigment produced by the DNA in this kit will be a lovely shade of pink that you can see under regular light and fluoresce under blacklight. The DNA was created by the Dean Lab at Stony Brook University in New York.

You can learn more about the DNA in this paper:
A New Purple Fluorescent Color Marker for Genetic Studies in Saccharomyces cerevisiae and Candida albicans by Sabine Keppler-Ross, Christine Noffz, Neta Dean
https://academic.oup.com/genetics/article/179/1/705/6064800



Yeast Engineer-it Kit Metabolic Selection - Pink DNA. Results after 48 hours of incubation under natural light (left) and under blacklight in a DIY blacklight photobox (right).

To summarize, your kit will allow you to complete the following hands-on steps to insert a DNA plasmid into yeast. This process is also called a “transformation”:

1. **Make selective and non-selective agar plates for growing yeast**
2. **Streak/grow blank (non-engineered) yeast**
3. **Make yeast cells chemically competent (able to take up DNA plasmids)**
4. **Transform the competent yeast with DNA program**
5. **Recover the engineered yeast**
6. **Grow the engineered yeast on plates**
7. **View the results**

Kit components

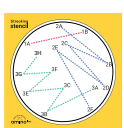
If you have the Group kit, you'll find the items with the ** below in the shared materials bag.

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 SC agar powder is enough to make 4 SC agar plates.[1]

Blue Loops: Small inoculating loops are used for transferring 1 uL of liquid and mixing. uL stands for µL which means microliter, so one-millionth of a liter. These replace costly traditional pipettes.

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

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



Streaking Stencil: To grow the blank yeast into separated, fast-growing colonies for engineering, a specific streaking pattern that dilutes the number of yeast you initially have on your loop must be followed. This stencil will help you trace the pattern when you place it under a petri dish.

Inactivation Bag **: A heavy duty bag to put opened tubes, used loops and petri dishes in. After the experiment is completed, simply add bleach and water to the bag to inactivate all the material and practice safe science as per *Storage, disposal & clean up* Instructions.

Day 1 bag (used on the first day of the experiment)

Agar Powder: This tube contains a carefully determined mix of nutrients and agar powders called SC agar. This mixture is a food source for yeast that can make their own uracil, thereby "selecting" for these types of yeast. SC agar is therefore selective agar and it creates the solid surface the yeast will grow on. Your SC agar will be packaged in either 1 yellow-top tube or 2 white-top tubes. Depending on which you get, add 1 or both tubes when you are making your SC agar plates.

Day 1 bag (cont.)

N.S. Nutrient: This tube contains a rich mixture of nutrients in powder form that acts as food for yeast, and converts the SC selective media to non-selective media to grow your blank yeast on before your engineer them with the DNA.

Blank yeast **: This safe, standard W303A lab strain of yeast is non-pathogenic and is used by thousands of labs around the world. These yeast come as a "stab," which is a small tube containaing soft agar in which the yeasts are grown.[1] It feels like a soft jelly substance and that's normal!

Day 2 bag (used on the second day of the experiment)

Transformation Buffer: Amino Labs' proprietary transformation buffer is used in a colony transformation procedure to yield high transformation efficiencies. When you adhere strictly to the transformation protocol, this buffer rivals other commercially available competent cells & procedures that are costly and require specialized laboratory equipment to store.[1]

Booster: Amino Labs' proprietary transformation booster is used in the colony transformation procedure to yield high transformation efficiencies.

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]

+ Cells **: This stab of engineered *yeast* is non-pathogenic and allows you to create a positive control sample by growing it on a plate to test if your selective agar plates were successful.

DNA **: This tube contains DNA plasmids which you will use to program your yeast so that they create coloful pigment and survive on selective plates.

[1] For education purposes only.

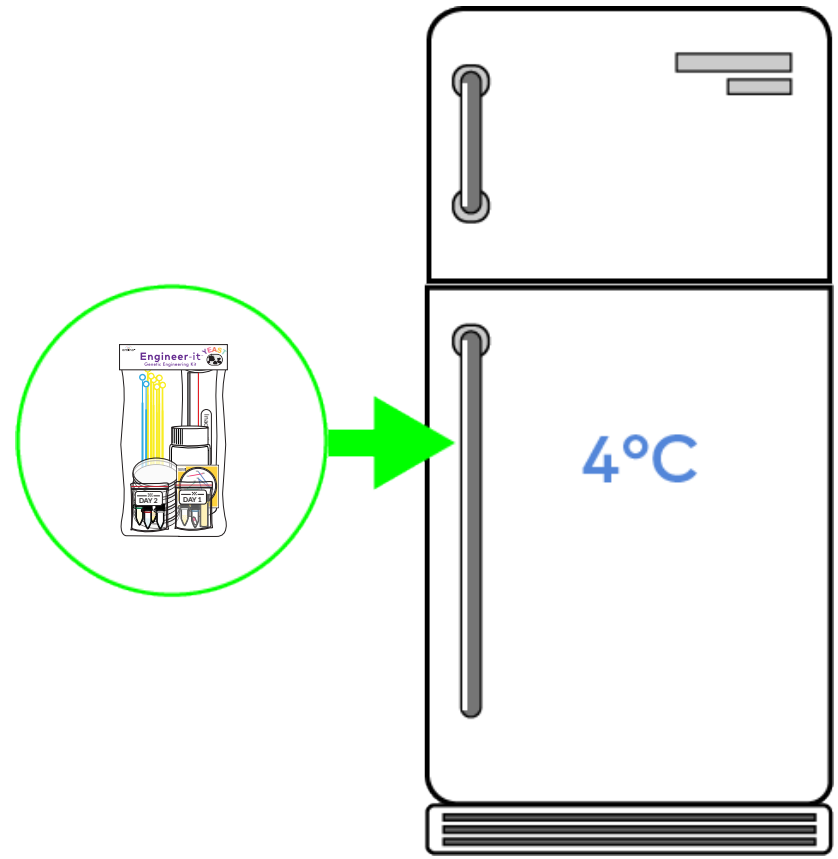
Unpacking and storing kits

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

If you can fit the whole pack, go ahead and store it all in the refrigerator. If you need to save space, please put the DAY 1 and DAY 2 bags in the refrigerator. The rest can stay at room temperature.

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!



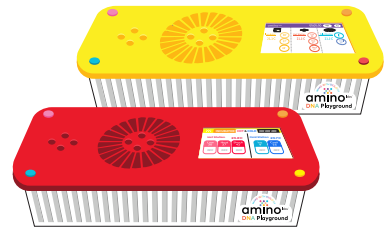
Technical specs

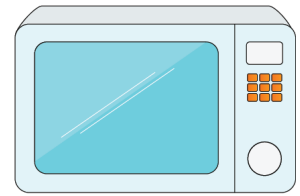
Growth plates: 6 cm petri dishes	Transformation Buffer: 50 uL tubes	Solid growth media:
DNA plasmid 250 ng	Recovery media: 350 uL tubes	SC agar powder (1.6 g)
Selection: YPD Broth	Cells /+Cells: Yeast Stab	50 mL sterile water

Necessary equipment

For Best results:

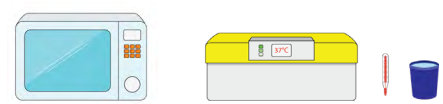
- **DNA Playground:** One DNA Playground Classroom size per 4 experiments running at the same time or one DNA Playground Home size per experiment
- **Microwave**
- **1 Sharpie-type marker**





Alternative solution:

- **Microwave**
- **Hot water bath or bowl with hot water**: This will become your **Hot station** set to "**Shock/42**" for the experiment. Heat the water to 42°C and try to keep it as stable as possible while you heatshock.
- **Thermometer (**for 42°C**)**
- **Timer**
- **Incubator (**for 30°C**)** : This will replace the **Incubator** set to "**30**". If you do not have an incubator (biology or egg one, as long as they set to 30°C), you can create one using an online tutorial Search for DIY incubator on our youtube chanel - Youtube.com/aminolabs - or go to this direct link: https://www.youtube.com/watch?v=LEsv0Qvbczs



Necessary safety supplies

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

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

4-day (period) timeline

The timeline of the Engineer-it Kit™for Yeast is made up of 4 days of hands-on activity and 24 to 72 hours before seeing results.

Six main steps make up the Engineer-it Yeast experiment:

1. Make selective and non-selective SC agar petri dishes
   Day 1, 25 minutes

2. Streak/Grow blank yeast cells
   Day 1, 20 minutes + 16-25 hours incubation MAXIMUM

3. Make yeast cells chemically competent
   Day 2, 20 minutes

4. Engineer competent yeast cells with DNA & start recovery
   Day 2, 30 minutes + 12-24 hours recovery*

5. Plate recovered, engineered yeast cells
   Day 3, 30 minutes + 24-72 hr incubation

6. View results
   Day 4, 20 minutes

* if you want to complete your experiment in 3 days instead of 4, you can shorten the recovery step from 24 hours to 30 minutes before proceeding to Step 5 on the same day.

3 key pitfalls to avoid!

In the next pages are detailed, step-by-step instructions to complete the experiment and genetically engineer yeast with DNA. **Please make sure you 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 SC agar, make sure that the water is boiling before adding the agar powder. **You have to see the water bubbling! Also, after mixing in the agar powder, microwave once more until you see boiling/ foaming.** Caution, the bottles will be hot!

If you have completed other Engineer-it Kits, note that with the SC agar, you pour your 3 Selective petri dishes first, before adding the N.S. Nutrient powder that turns your SC agar into Non-Selective agar so you can pour your last petri dish, the N.S. petri dish. You'll learn more about why this is in the next pages.

2. In Step 2: **After streaking the blank cells, the colonies should be used no later than 25 hours after streaking.** Otherwise, your experiment will not work.

3. In Step 4: When adding the DNA to the tubes of competent cells, make sure you see liquid in the loop before adding it to their tube. Make sure to twist the loop in the liquid for at least 5 seconds to ensure the DNA mixes in.

Experiment Protocol

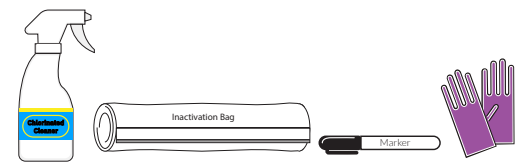
0. Prepare your space

Goal Set yourself up for sucess.

Materials from the kit
(1) Inactivation bag

Materials not in your kit
(1) 1L discard container
Chlorinated bleach spray or wipes

Paper towels
(1/person) Pair of gloves



Make sure you have the necessary materials as explained on page 13, including gloves, microwave, and cleaner before you start and that you have read and understood the safety guidelines. You can download and print a safety checklist to complete before and after your experiment from www.amino.bio/checklist

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

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

- your tubes of cells or DNA if you are not saving them for a future experiment*,
- any used inactivation loops (the paper/plastic sleeves can go in normal garbage),
- blank cell petri dish once they have been used to create competent cells
- any empty tubes like the agar, buffer and selection tubes (with the lids removed!),
- any gloves that have touched yeast.

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

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

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

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

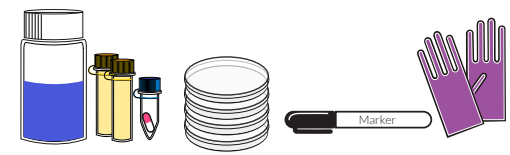
Student's Experiment Protocol

1. Creating SC Agar Plates Day 1, 25 minutes

Goal Create non-selective and selective SC agar plates.

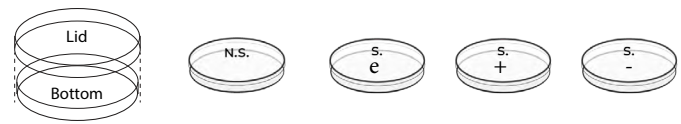
Materials from your kit

(1) 50 mL sterile water
(1) N.S. nutrient tube
(4) 6 cm petri dishes
(1) Sharpie marker
SC agar powder:
1 yellow-top tube or 2 white-top tubes



Prepare

1.1 Label the bottom of the petri dishes with a marker: **1x** N.S. [your initials] **3x** S.[your initials]. Of these three, label one "**+**", one "**-**", and one with an "**e**". (*The bottom is the part that has the smaller diameter of the two: the bottom fits inside the lid)*



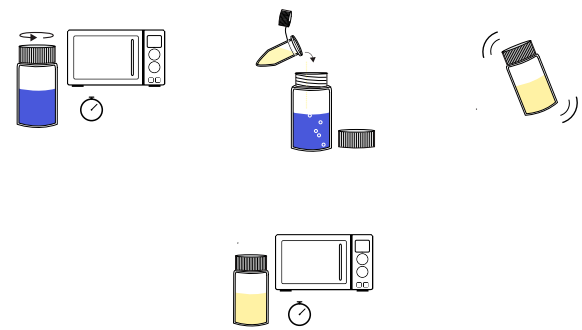
Mix the Agar

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. **!! If the water does not boil, the agar powder will not dissolved and your plates will not solidfy !!**

1.4 Add the SC agar powder to the boiled water. Careful, the bottle will be hot! A bit of agar powder may clump around the lip of the tube due to water evaporation. That's okay! we've accounted for this possible loss. Your SC agar is packaged in 1 yellow-top tube or 2 white-top tubes; add all the SC powder you have.

1.5 Microwave the water and agar powder in 4 seconds intervals until you see it boil again (it may look like foam is forming at the top). ***Careful, the liquid will boil over and spill if you microwave in more than 4 sec. increments.*** After you see the liquid foam, remove from the bottle from the microwave and swirl to mix for 10 seconds.

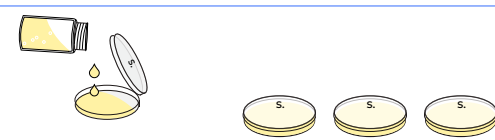


1.6 Repeat this step one more time, then swirl to mix for 30 seconds or until you see all powder dissolved.

Make selective (S.) plate

*** Read p.9-10 to learn why you are pouring your 3 selective petri dishes first!***

1.7 Pour molten SC agar in the bottom half of your "+", "-", and "e" Petri dishes, enough to fill the bottom petri dish half-full. Place the lid ¾ of the way back on to let the agar can cool and dry (solidify).



Make non-selective (N.S.) plate

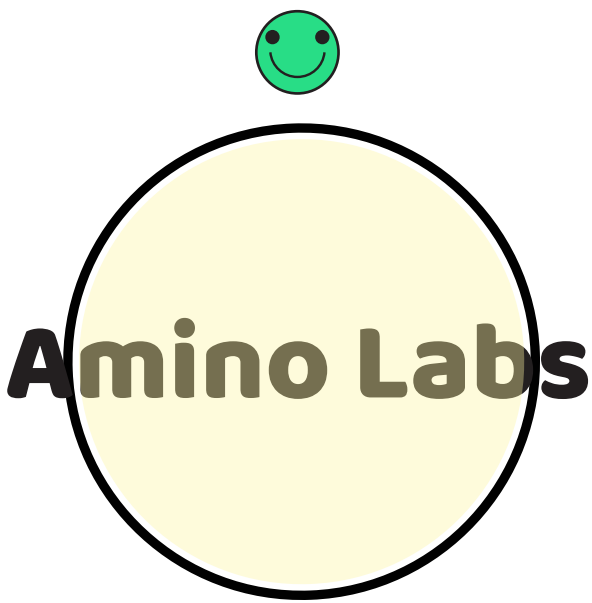
1.8 Pour the content of the N.S. Nutrient tube to the bottle of molten agar and gently swirl for a few minutes until the contents of the have dissolved. Try not introduce bubbles into the agar don't swirl too vigorously!

1.9 Once all the powder is dissolved, pour the molten agar into the bottom of the remaining petri dish marked N.S. and place the lid ¾ of the way back on for the agar to cool and dry (solidify).

1.10 Let the agar harden. The non-selective (N.S.) 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

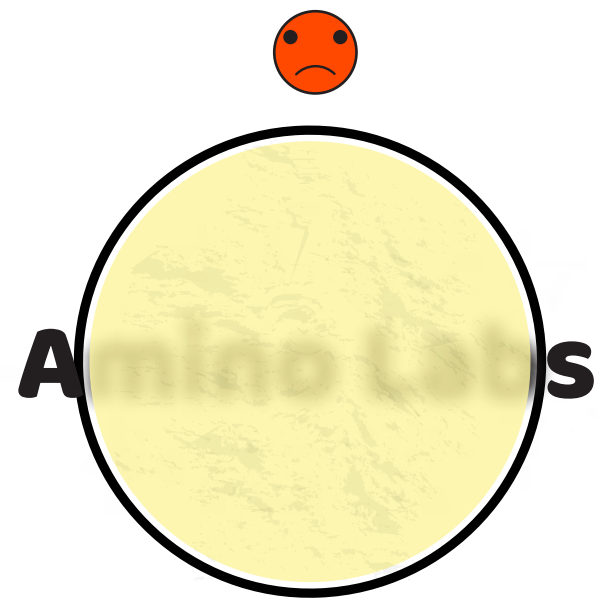
Checkpoint - Agar Plates

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



A perfect Agar petri dish, also called an 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 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 plates.

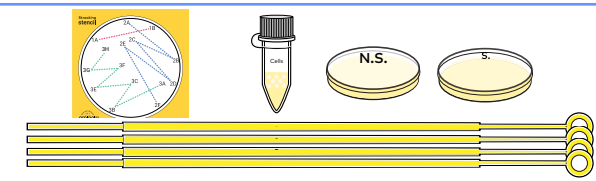
Unfortunately, if the agar does not solidify, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

2. Growing Blank Cells Day 1, 20-45 minutes + 12-25 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

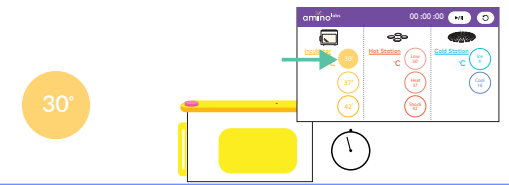
Non-selective & "-" selective plate
(1) Plate streaking stencil
(4) Yellow Inoculation Loops
(1) Stab of blank cells ***(Not Cells +)***



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 30°C



Streak your Plate

2.2 Place your N.S. Petri dish on top of the Plate streaking stencil. Take one yellow loop and dip it into the stab of blank yeast cells. Inspect your loop to make sure it appears wet to confirm you have collected cells.

2.3 Open your petri dish, and trace the line 1of the stencil on the surface of the agar with this loop. You can trace it back and forth a few time to deposit a lot of cells on this line. Discard the Loop in the discard container.

2.4 Using a new yellow loop, trace line 2 only once. Discard the loop.

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

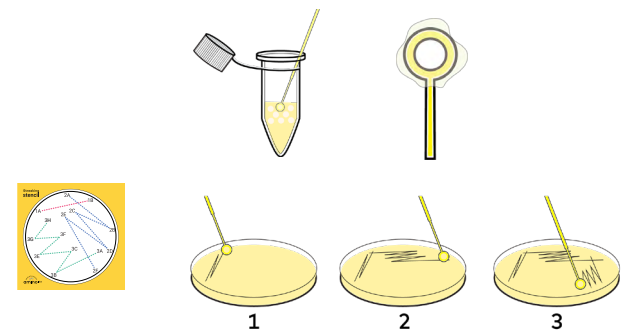
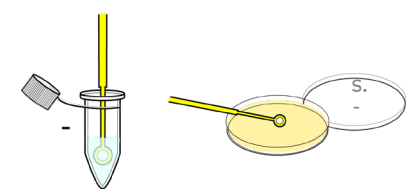


Plate your negative control ("-" plate)

2.6 Take your Selective agar plate labeled "(-)". Using a single yellow loop, dip into the blank cells and spread them across your agar plate in any pattern of your choosing. This is your negative control. On this selective "-" plate, the blank cells should not grow.

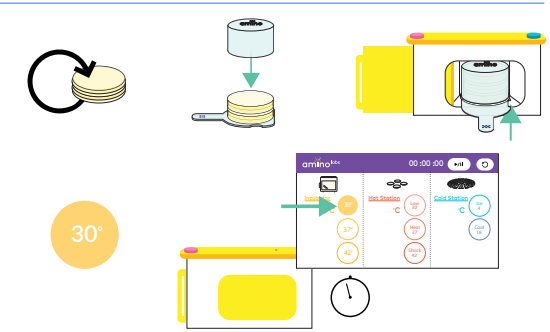
2.7 Close your tube of yeast and place it back in the fridge in a ziploc bag if you want to keep them in case anything goes wrong or discard them in the Inactivation bag if you do not want to save them.



Incubate Overnight

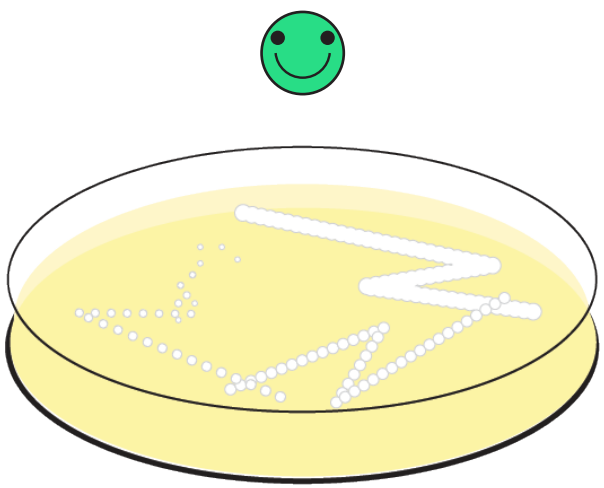
2.8 Flip your streaked N.S. & S. "(-)" plates upside down and stack them in a pile in an incubator set to 30°C. If you are using the DNA Playground, put your stack of plates on top of the incubator paddle, set the incubator humidity chamber on top and slide in the incubator. Close the incubator door and lock it using the incubator key.

It is important to do the next steps in 12 to 25 hours so that your cells will be in their optimal growth phase. If you wait longer, your experiment will not work. Note that with yeast it will take closer to 24 hours to see colonies.

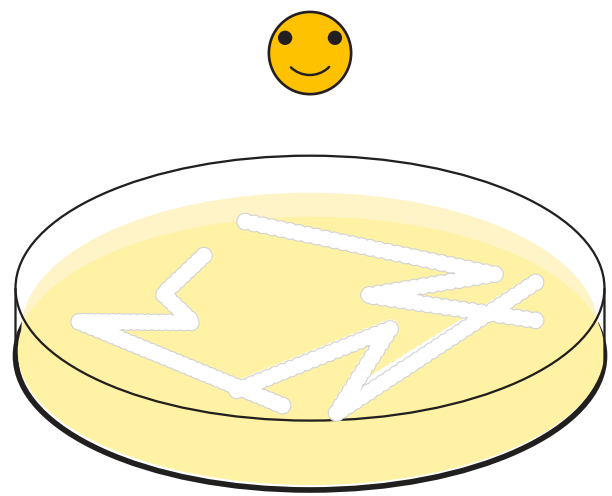


Checkpoint - Non-selective plate & blank cells

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

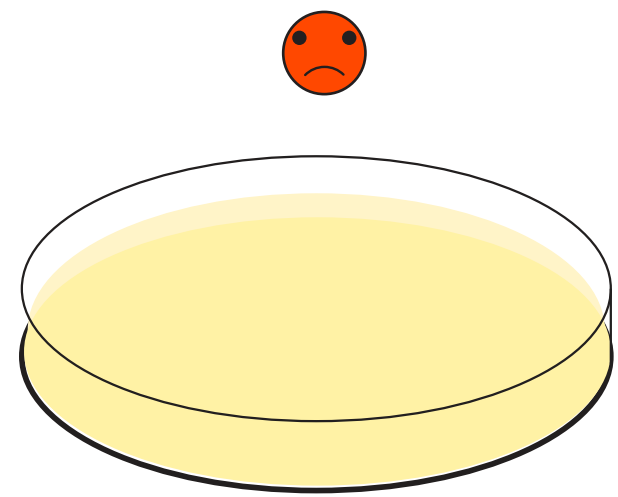


A perfect N.S. plate has lots of small individual white colonies (dots) of fast-growing yeast after incubation. Proceed to the next page.



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

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:

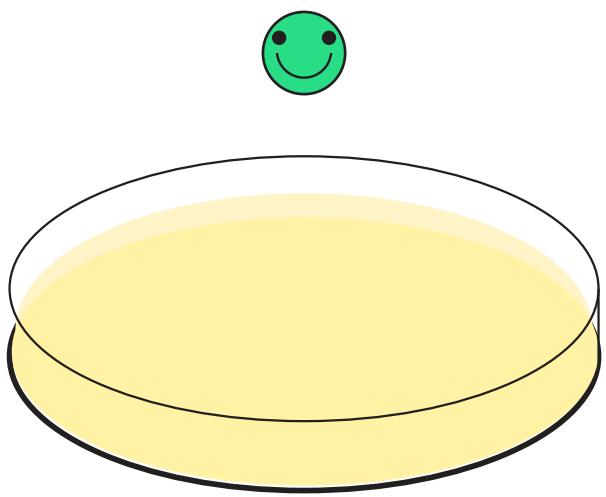
1. If your incubator was not at 30°C or is homemade, incubate for another 24hrs.

2. If you are certain you incubated at 30°C, or incubated for 48hrs and still have no colonies, you might not have had cells on your loop when you streaked. Repeat Step 2: Growing Blank cells on this plate.

3. If you still have no colonies after repeating Step 2, complete the guide at www.amino.bio/troubleshoot

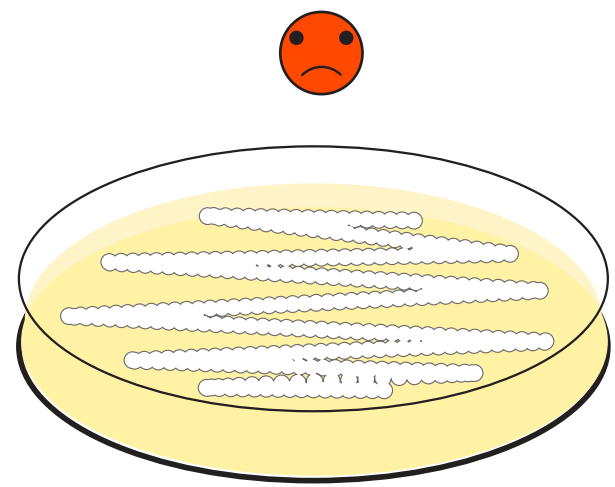
Checkpoint - Selective plate and “-” control

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



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

Move on to the next step.



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

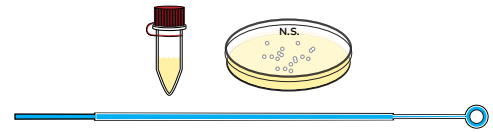
Continue the exercise with your other selective plates. Note that your results may not be as good as if you had the right amount of antibiotics. Don’t worry though, practice makes perfect, and our Success Guarantee means you will have the chance to be successful if this try doesn’t work.

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

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

Materials from your kit

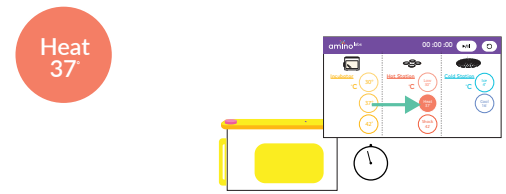
(1) Streaked N.S. Plate
(1) Blue Loop
(1) T. Buffer Tube



Prepare

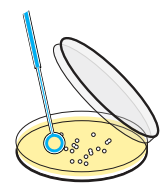
3.1 Make sure you have completed the Non-Selective Plate and "-" control Checkpoints. You can discard the "-" plate in your discard container.

3.2 Turn on the "Heat 37 °C" setting on your DNA Playground™ or get your warm water bath.



Mix the cells and T. Buffer

3.3 Take your T. Buffer tube and make sure all the liquid is in the bottom of the tube by tapping it gently on the table surface. You should have approximately 5 mm of liquid in the bottom of the tube. Place it in a tube holder or on the Cold Station in the off position (room temperature).

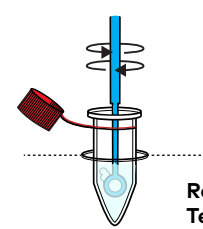


3.4 Take a blue inoculating loop and gently scrape it over small, well-separated colonies on your N.S. plate. Colonies that are ~1mm in diameter work the best. You want to collect ~10 or 20 of these colonies on your loop, enough so that you can see that the center of the loop is full.

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

3.5 Immerse the loop with the yeast in the Transformation Buffer without touching the sides of the tubes.

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



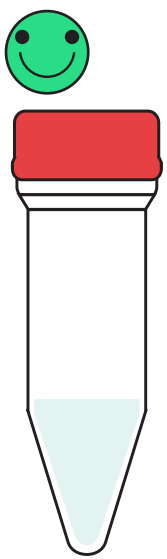
When successful, the solution should be cloudy, and there should be no clumps floating in the solution. You may have to mix vigorously for 10-30 seconds. If you see clumps, keep blending.

This step should take no more than 2 minutes! You need to move on to the next step of adding DNA quickly!

Do not throw out your N.S. petri dish. Place it back in the ziploc bag and in the refrigerator when you have time.

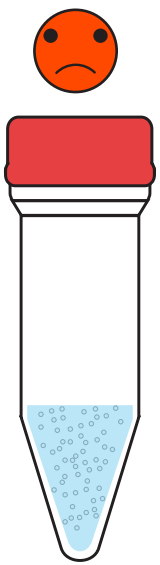
Checkpoint - Competent cells

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

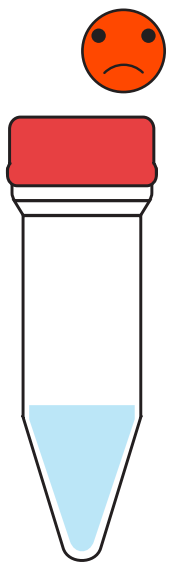


Cloudy liquid - Perfect!
The cells can now take up DNA.

Move on to the next step.



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

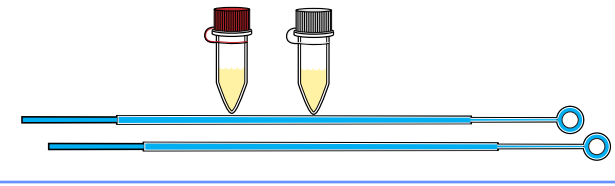


Clear liquid? Add more cells and mix until the liquid is cloudy like the image on the left.

4. Adding your DNA Day 2, 20 min.

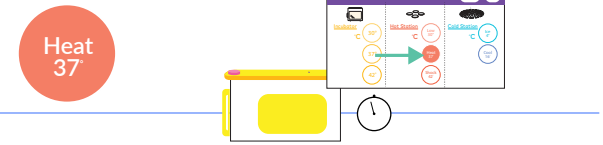
Goal Introduce a DNA plasmid into competent yeast and start recovering the cells.

Materials from your kit
(1) DNA plasmid tube
(2) Blue Loop
(1) Competent Cells {from prior step}



Prepare

4.1 If it is not alreay on (it should be!), turn on the "Heat 37 °C" Hot station setting on your DNA Playground™ as you will need it at the end of this step. Keep your transformation buffer tube at room temperature for now.



Mix the competent cells & the DNA

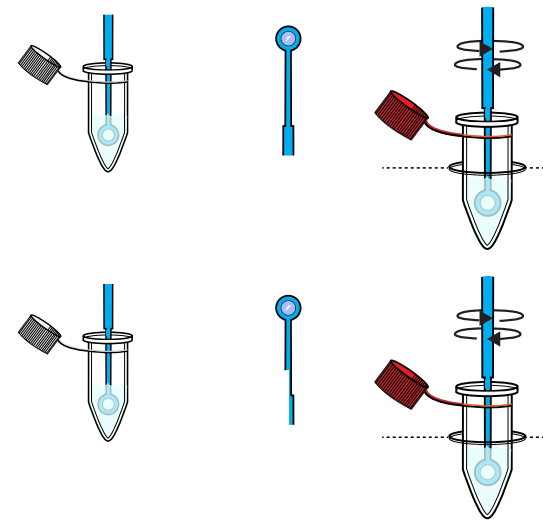
4.2 Dip a blue inoculating loop into the DNA tube and twist a few times. When you pull the loop out of the DNA tube, the hole of the loop should have liquid in it. This is the DNA you will use to engineer your cells. Make sure you can see liquid in the loop!

4.3 Slowly dip and spin the inoculating loop containing DNA into the tube of competent cells you made in the previous step. Stir/swirl for 10 seconds to fully mix. Discard the loop. * Do not reuse the inoculating loop! *

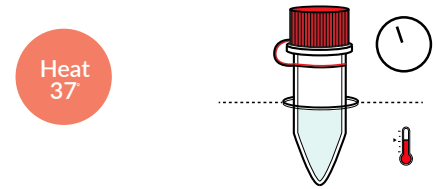
4.4 Using a new blue loop, repeat adding DNA to your tube of competent cells by following steps 4.2 and 4.3 again.

Note: After this step you will have added a total of two 2 uL (two blue loops) of DNA.

4.5 Incubate your tube for 5 minutes on your Hot Station set to 37 °C.



Remember! Do not throw out your N.S. petri dish. Place it back in the ziploc bag and in the refrigerator when you have time.

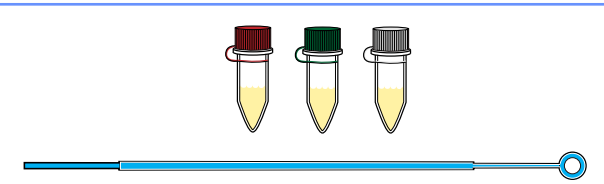


5. Booster, HeatShock & starting the recovery Day 2, 35 min.

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

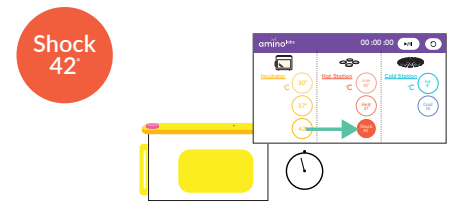
Materials from your kit

(1) Competent Cells with DNA {from prior step}
(1) Recovery Media tube
(1) Booster tube
(1) Blue loop



Add the Booster

5.1 After 5 minutes at 37 °C on the Hot station, increase the temperature of the hot station (or your water bath) to “Shock 42 °C” setting.

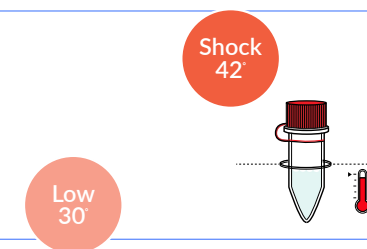


5.2 While the temperature rises, you will add your T. Booster to the tube of cells and DNA: Using a new blue loop, dip it into the T. Booster tube and twist it to collect liquid in the center of the loop (just like when you added the DNA). Inspect the loop to ensure that some T. Booster is in the loop centre.

5.3 Insert the loop with T. Booster into your tube of cells +T. buffer+DNA. Twist to mix it in for 10 seconds.

HeatShock

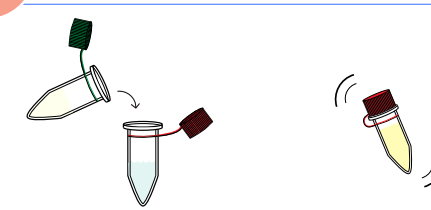
5.4 Incubate the tube with T. Buffer, cells, DNA, and T. Booster at 42°C for 15 minutes. This is the heat shock step.



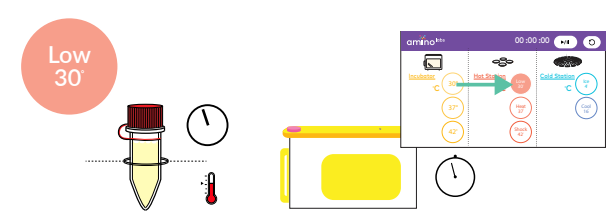
5.5 After the 15 minute is up, turn your DNA Playground’s Hot station to ‘Heat 30°C’ or adjust your water bath temperature to 30°C. The temperature on the DNA Playground will cool to the correct temperature over the next 15 minutes. There is no need to wait for it to cool, just continue on to the next step.

Recovery

5.6 Pour the tube of Recovery media (~350 uL) into your tube of Transformed cells. Mix gently by inverting 10 times. Some Recovery media liquid will stay in the recovery tube. That is acceptable.



5.7 Place your tube with your Transformed cells in your DNA Playground’s Hot station or water bath that is set to 30°C. If it is still a higher temperature that is OK, just allow it to cool with the tube in the station. This temperature transition is designed into the procedure. Double check to make sure the liquid is in the bottom of your tube, not on the sides. See www.amino.bio/whip-it to learn a fun technique, the Whip-it method, for moving liquid inside a tube.



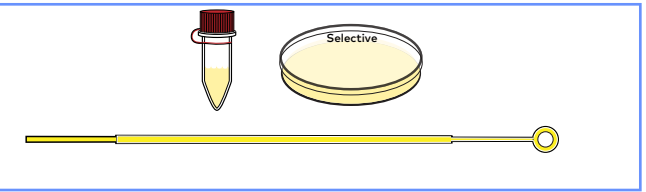
5.8 Leave your tube to recover at 30°C for up to 24 hours (minimum of 30 minutes, with best results after at least 12 hours). If possible, briefly shake/mix the cells a couple times over the period. This step allows the Transformed cells to recover and start expressing their new DNA programs.

6. Plating Cells Day 3, 20 minutes + 24-72 hours wait

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

Materials from your kit

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



Prepare

6.1 After your 30 minutes to 24 hours of recovery period, turn on your Incubator at 30°C. Note that it can take up to an hour to reach 30°C, but you can place your petri dishes in the incubator before it reaches temperature.

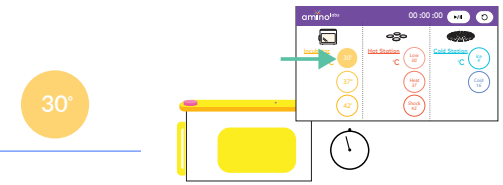


Plate your transformed cells ("e" plate)

6.2 On the Selective agar plate labeled "e", pour 1/2 of your recovered Transformed cells 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 the rest of your Transformed cells in a fridge and if you do not see any colonies of engineered cells after 48 hours incubation, you can pour the rest on the plate and incubate again. You can also discard your tube of recovered cells by removing the lid and placing them in the inactivation bag.

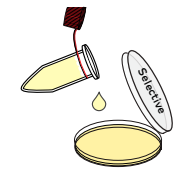
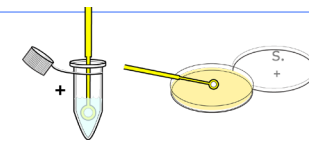


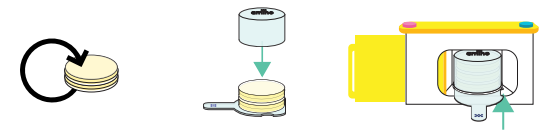
Plate your positive control ("+" plate)

6.3 Get the stab of "+ cells" and your Selective 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.

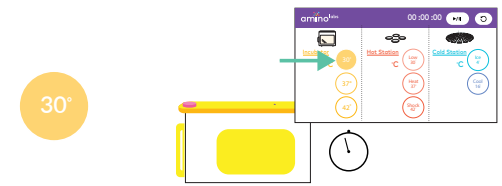


Incubate

6.4 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 agar surface moist.



6.5 Incubate for 24-72 hours until the trait is expressed (for example, until you see a soft pink color). Swap the order of the plates, (i.e. the top one goes to the bottom and the bottom one to the top) every 24 hrs.



7. Did your cells grow? Day 4+

Goal **Verify if you have any "e" cells that have grown and are producing your specific DNA Program.**

Your engineered yeast will grow in colonies and start producing their new DNA program in the next 24+ hours. Yeast grow slower than bacteria, so it may take uo to 48 hours before you see anything on your petri dish. Keep an eye out, and your camera ready to document!

Remember that getting even a single colony is a success! Many scientists doing research often hope for a single colony! If you get more than one colony, this means you followed the procedure very well. If you repeat this experiment, you will very likely get more colonies than you did this time, because you will have practiced the procedure and like most things in life, practice makes perfect!

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8. Seeing your results.

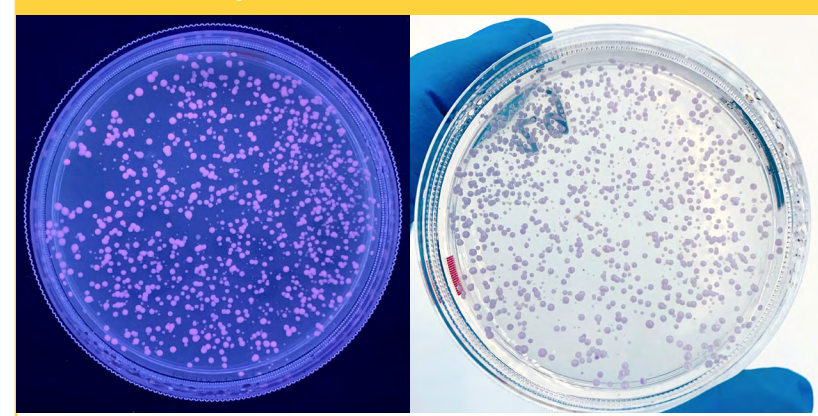
As your engineered yeast grow, they will also start producing the DNA program that tells them to create a colorful pigment. The pigment in your kit is visible in regular light and fluoresces under blacklight/UV light. While yeast grow slowly and will start growing white, as they grow and you continue to incubate them, you will eventually see nice pink color!

It can take up to 72 hours to see the color brightly. You can take out your blank yeast cells (plate N.S.) to compare it to your engineered yeast cells (plate S. (e)) to see the difference your DNA plasmid made!

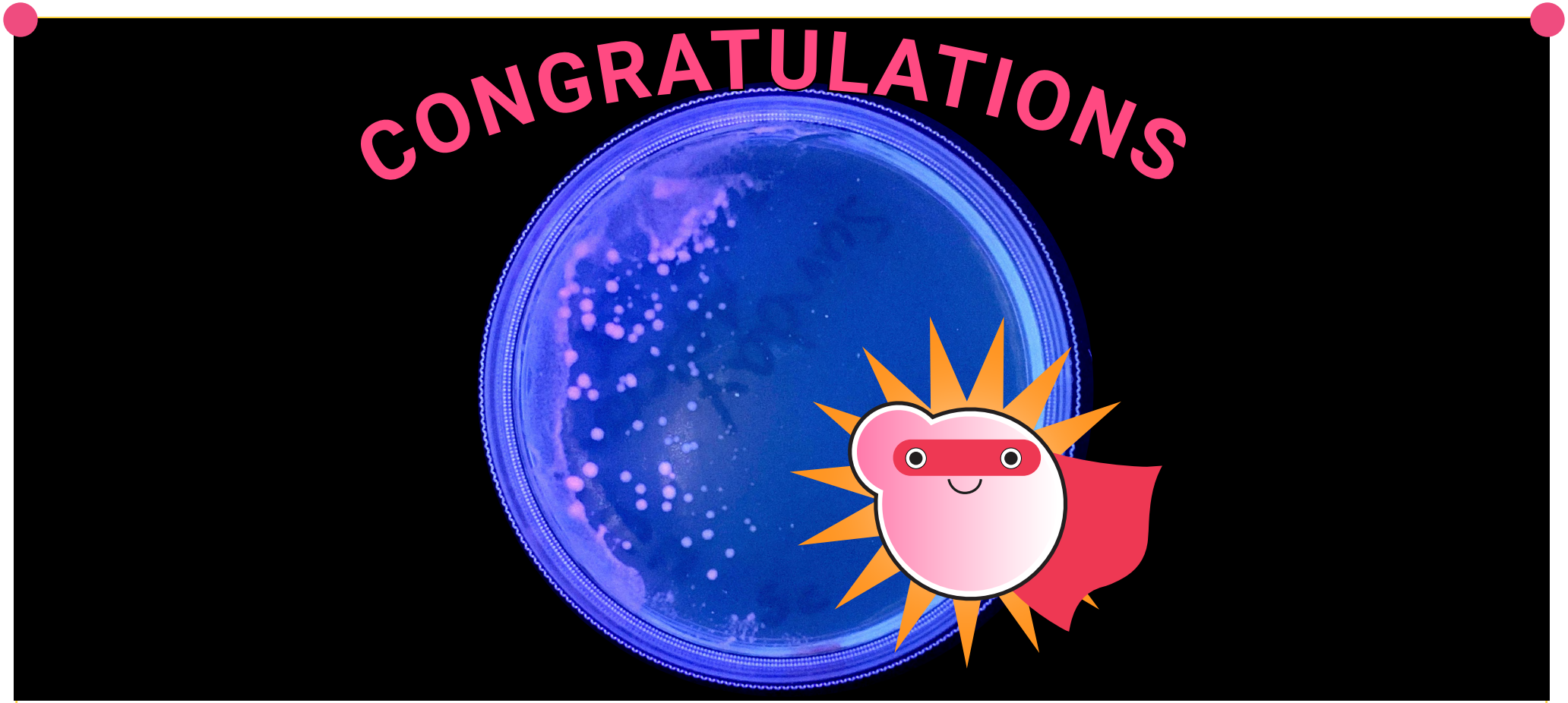
Your positive control: "+ cells" should be visible if your selective plates were properly made. The + cells are also engineered yeast cells that will produce the color pigment. These will develop color much faster than your newly engineered cells.

Problems? If you cannot see any growing cells at all after 48 hours, your experiment may have failed. Don't be discouraged! In science, failure is a chance to learn more. Complete the troubleshooting guide at www.amino.bio/troubleshoot to get a new chance to try the experiment, thanks to the success guarantee!

What to expect



Yeast engineered with the Pink DNA after 72 hours of incubation. Photographed in the blacklight photobox (left) and under natural light (right)



You did it! Using your Yeast Engineer-it Kit™, you inserted a DNA Program inside single-celled organisms called yeast and had the yeast execute that DNA Program to produce something for you, just like scientists and industries do every day inside their large laboratories. Congratulations!

We hope you enjoyed the experience and will continue to experiment in the field of genetic engineering. You can preserve your results with our Keep-it Kit™. Show off your results with your friends, our community and us too! We'd love to see your work - find us on Twitter, Instagram, Facebook, and Youtube under aminobiolab.

For now, let's make sure you dispose of and store your remaining material correctly.

Storage, Disposal, Clean Up

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

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

B. Reusable materials: If you have DNA in your kit, it can last up to 6 months when stored in a refrigerator. If you wish to keep it, store it in a ziploc bag inside a sealed plastic container in a refrigerator away from food items. If you do not wish to keep it, add to an inactivation bag. Make sure the lids are separate from the tubes so that the inactivating liquid can get inside. If you see any mold or unknown yeast 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 yeast growing inside, then you should always immediately inactivate the Petri dishes.

D. Inactivation: Make sure all yeast, 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 yeast go in the regular garbage or recycling.

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

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

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

Glossary

Nutrient Agar: A Jello-like substance that serves as a growth substrate for yeast. It is a mixture of nutrients, vitamins, minerals, and agar. Nutrient agar powder dissolves in boiling water and solidifies at room temperature, creating a soft surface your organisms can grow on and use as a source of food. There are different types of yeast agar. A common one is YPD agar. YPD is made up of dead yeast, vitamins, and minerals. YPD can also be found in liquid-form (without the agar!). Another agar you will use with yeast is SC-Ura agar. SC-Ura agar is a minimal media or minimal agar, which is usually made to be missing one of the nutrients yeast needs to grow, uracil.

Antibiotics: When you engineer yeast using antibiotic-resistance, they become resistant to a type of antibiotics called G418. This antibiotic will be mixed in with the nutrient agar so that, as you incubate your culture, only transformed, G418-resistant yeast 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 121 °C) for several minutes, up to an hour. Autoclaves are similar to some baby bottle sterilizers or pressure cookers 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. Yeast 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 yeast can only be seen with a microscope, but they reproduce so rapidly that they often form colonies that we can see. Yeast reproduce when one cell splits into two cells through a process called binary fission. Fission occurs rapidly, in as little as 60 minutes.

Competent Cells: Since DNA is a very hydrophilic molecule, it won't normally pass through a yeast cell's membrane. In order to make yeast 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 yeast cells by suspending them in a solution with a high concentration of lithium (the transformation buffer). DNA can then be forced into the cells by incubating them briefly at 42°C (heat shock). This causes the yeast to take in the DNA and is called "Transformation".

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

DNA plasmid: A plasmid is a small circular piece of DNA (about 2,000 to 10,000 base pairs) that contains essential genetic information for the growth of yeast. Yeast share vital information by passing it among themselves in the form of genes in plasmids. By inserting a new plasmid in our yeast, 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 the genetic material of an organism. It consists of DNA. Learn more about genomes in the *What is DNA?* simulator on amino.bio

Heatshock: a heatshock happens when the cells are moved from an ice-cold temperature to a warm temperature, typically 42°C, to help the cells take in DNA plasmids more efficiently.

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

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

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

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

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

Selective: A selective plate contains antibiotics (antibiotic selection) or is deficient in a nutrient that the organisms need to grow (metabolic selection).

Selection, Antibiotic: With antibiotic selection, you add antibiotics to your media (usually your nutrient agar) to create a selective media/selective agar. Then, as you insert a new DNA plasmid into cells to make them create something for you (like a color pigment) you are also adding a 'selective marker' (antibiotic-resistance gene) coded in the same DNA plasmid. Adding the antibiotic-resistant gene to the cells means that only the cells that have taken up the new DNA will be able to "resist" the antibiotics and be able to grow on an agar petri dish that has the antibiotics mixed in. You are then 'selecting' for your engineered cells with the selective agar!

Selection, Metabolic: With metabolic selection, you create a media that is missing a key nutrient your organism needs to survive and grow. As you then insert a new DNA program into cells to make them create pigments (or something else), you are also giving them the DNA code (gene)that allows the organism to gain the ability to create the missing nutrient themselves. This means that only the organisms that have "taken up" (been engineered with) the DNA plasmid will be able to grow on the media that has a nutrient missing since the non-engineered organisms would not be able to create that missing nutrient and would therefor not be able to survive. In other words, only engineered yeast will grow on the selective agar.

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 agar powder

2. You might not have added all the powder from the tube, resulting in too much water vs. 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 36+ 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 30°C. If it is not, or if you are growing at room temperature, then it can take much longer to see the yeast 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 yeast 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 yeast/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 Yeast. 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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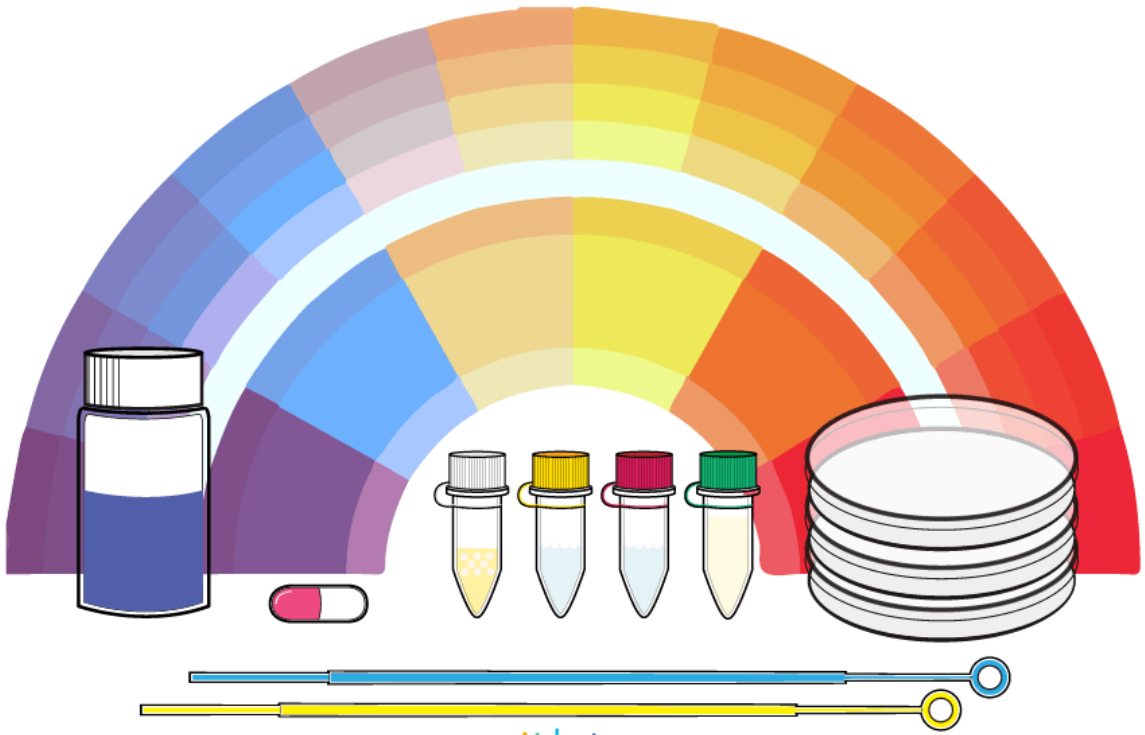
All Amino Labs products, from the hardware to the DNA, are invented, designed, manufactured and shipped by us, in our laboratory- workshop in Canada and we'd love to hear your feedback and suggestions to continue to make our products better and fitting to your needs. Answers to your questions and help are also just an email away.

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



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