MAKE WITH BIOLOGY

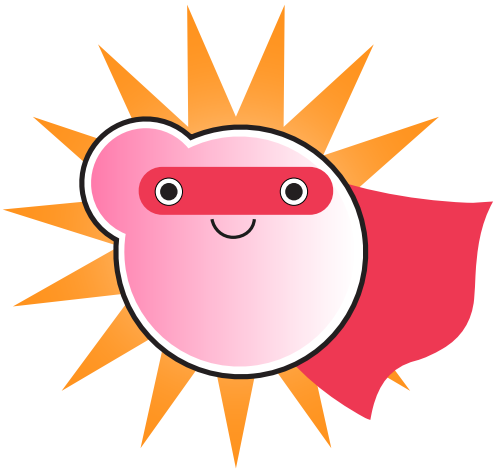
amino labs

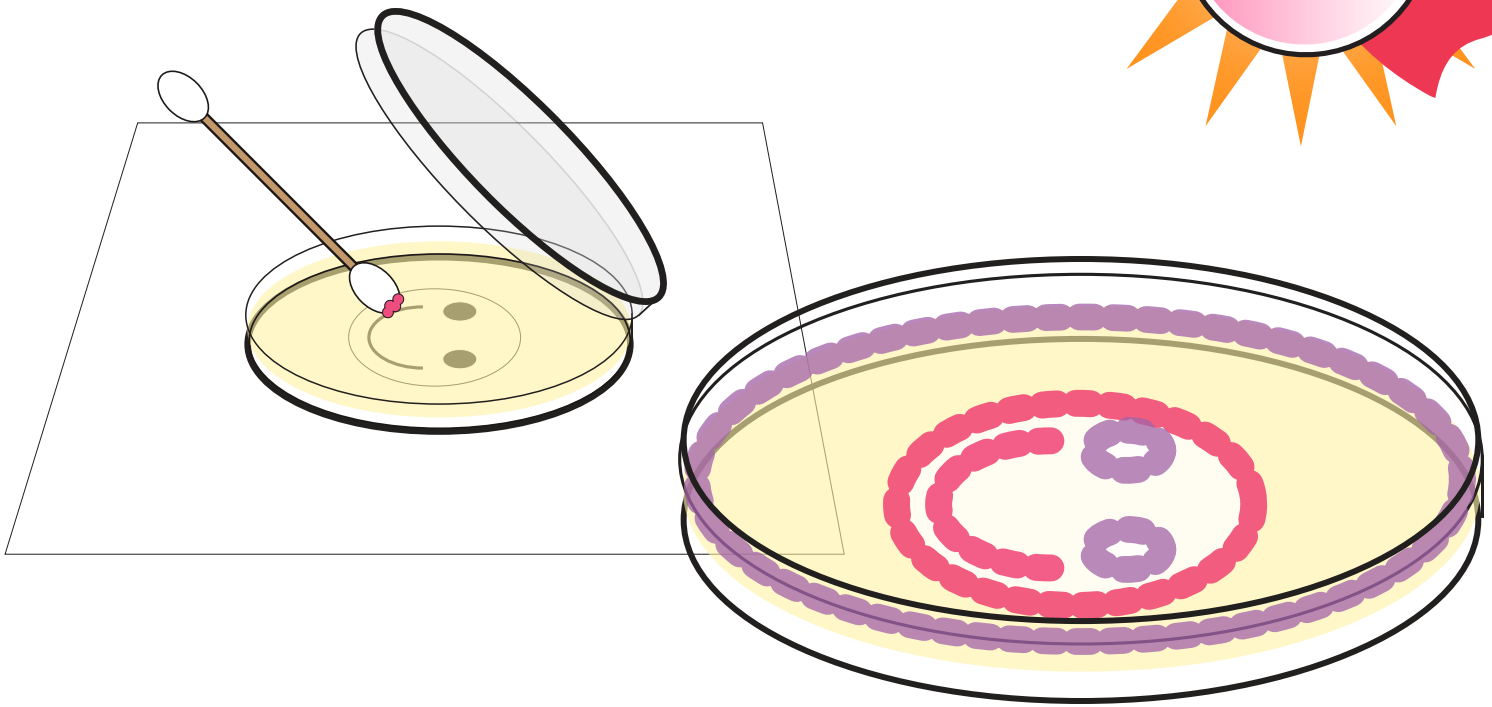
YEAST

CANVAS KIT™

MANUAL

Create living art with colorful yeast!





www.amino.bio

Version 2.1, Oct 2023

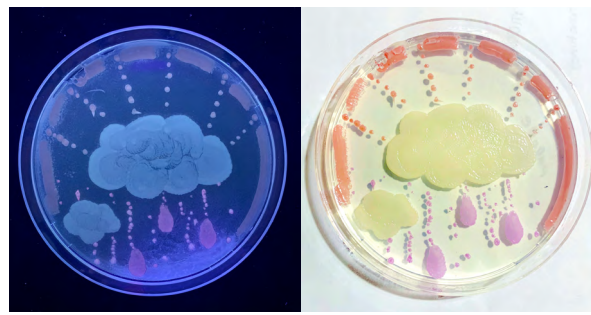
CANVAS KIT™

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 -- these are the step by step instructions on how to run your experiment. Make sure to follow our tips to ensure your best success! The third section covers "what's next"; how to keep your creations, store or dispose of any leftover ingredients and general clean up instructions. The final section is there to help you -- a glossary, troubleshooting, and our contact information.

Amino Labs is excited to welcome you to the world of the genetic engineering with the Canvas Kit™, Engineer-it Kit™ and our entire ecosystem of easy-to-use, easy-to-succeed at products! **Following this guide will help ensure that you are getting the most out of your current and future experiences 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), 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

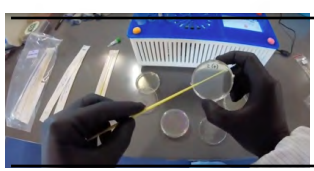
How will I learn?

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 Canvas Kit™

The Yeast Canvas Kit™ lets you use colored engineered yeast to create living paintings! By following the experiment instructions on the next pages, you will create selective agar petri dish “canvases”, use sterile paintbrushes”to create your living art on the agar surface and incubate over 24 to 72 hours to let your creativity grow!

The Canvas Kit comes in Individual Size or Group/Classroom Size. These different kit sizes contain the same ingredients, in different quantities. An individual size kits lets you create 3 paintings and one painting palette. The group size kits is separated in 8 student bags that lets each group create 3 paintings and one painting palette.

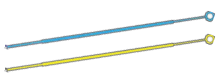
Note that the yeast and the agar are made to fit perfectly with eachother so make sure you keep kit components separate if you have more than one kit.

This kit can be used alone or in a small group (with parent supervision if you are 14 or under). In the next pages, you will find descriptions of the kit’s content.

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]



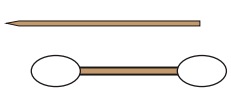
Inoculation Loops: Inoculating loops are used for transferring liquid, spreading cells on agar, and other tasks. In the Canvas experiment, you use the loops to spread your yeast paint on the agar in the petri dish.

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

Image stencil**: These stencils can be used as your art image. Place the stencil under the petri dish to trace.

Blank stencil: These stencils can be used to draw your bioart image before tracing it on the agar. Place the stencil under the petri dish to trace.

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 as per *Storage, disposal & clean up* Instructions.



Paintbrushes: Sterile swabs and picks to help you paint your yeast on the agar.

Day 1 bag

Agar Powder: This tube contains a carefully determined mix of nutrients and agar powders called SC agar. This mixture is a food source made specifically for yeast that can make their own uracil, like the yeasts you will be using in this kit. Since naturally occuring yeast cannot make their own uracil, SC agar is called a selective agar. Agar is both the food the yeast eat and the solid surface the yeast will grow on. Your SC agar will be packaged in either 1 yellow-top tube (1.6 g) or 2 white-top tubes (1 g each). Depending on which packaging you get, add 1 or both tubes when you are making your SC agar plates.

Yeast paint bag

Colored yeast paint** in pink, purple and white colors: These colored yeast are engineered to be colorful using safe lab-strains that are non-pathogenic.

[1] For education purposes only.

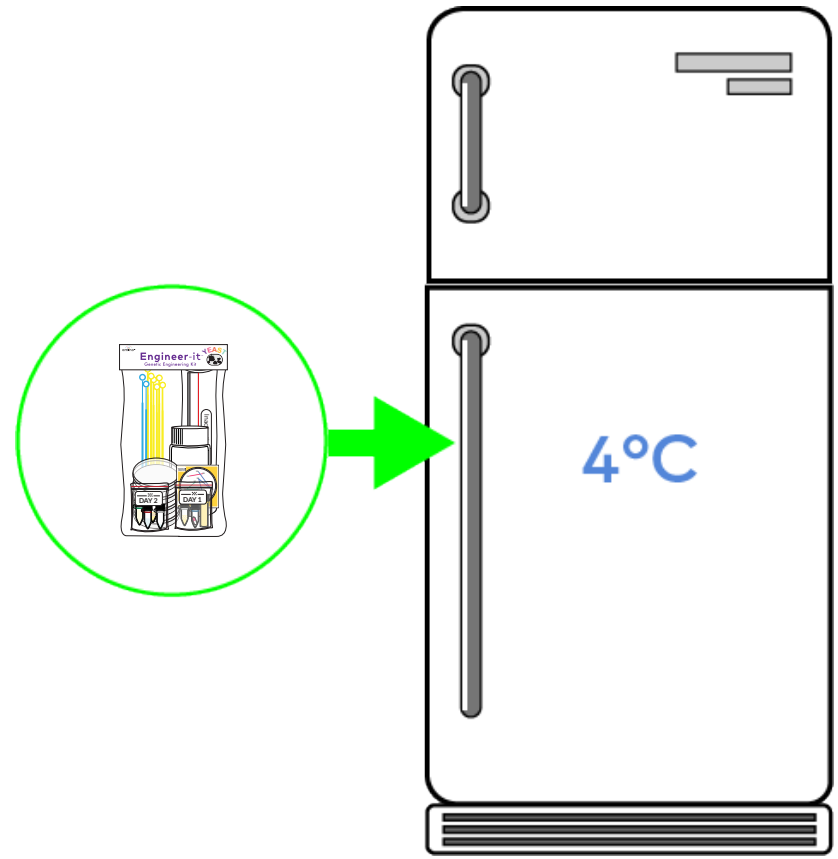
Unpacking and storing kits

For a better shelf life and successful experiments, place your Yeast Canvas 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 yeast paint bag 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
Paintbrushes & Loops

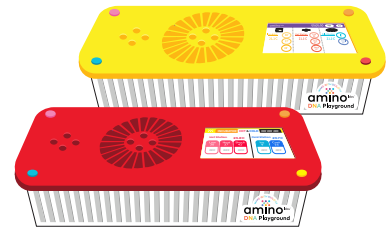
Paint: Yeast Stabs

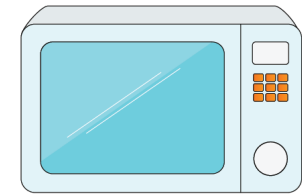
Solid growth media:
SC agar powder (1.6 g)
50 mL sterile water

Necessary equipment

For Best results:

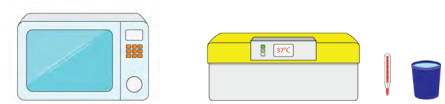
- **DNA Playground:** One DNA Playground Classroom size per 4 experiments running at the same time or one DNA Playground Home size per experiment
- **Microwave**
- **1 Sharpie-type marker**





Alternative solution:

- **Microwave**
- **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 Canvas Kit™ takes 2 days of hands-on activity to complete, and 48 to 72 hours to see results.

4 activities make up the Canvas Kit experiment:

1. Make selective SC agar plates
   Day 1, 20-35 minutes

2. Streak your colored cells to make enough paint
   Day 1, 20 minutes, incubate 24-48 hrs

3. Stencil your art on the blank stencils
   Day 2, 5-10 minutes +

4. Paint with your colorful yeast
   Day 2, 20+ minutes, 24-72 hrs incubation

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, you will have to microwave twice more until you see boiling/foaming.** Caution, the bottle will be hot!

If you have completed the bacteria Canvas kit, you'll notice that you do not need to add an antibiotic pill to this agar - that is because the SC agar is already selective since it is missing a key nutrient natural yeast need to survive: uracil. Your colored yeast, however, had been engineered to be able to produce the uracil it needs from other molecules present. This way, the SC agar is selective in that non-engineer yeast cannot grow on it. Learn more anout this type of selection by completing the Yeast Engineer-it Kit: Metabolic Selection.

2. In Step 3: **Before painting the art or flipping the painted petri dish upside down for incubation, make sure the agar is dry and there is no condensation.** Otherwise, the yeast paint may touch the condensation/water on the surface of the agar and spread around the agar. This means the painting will turn out very blury!

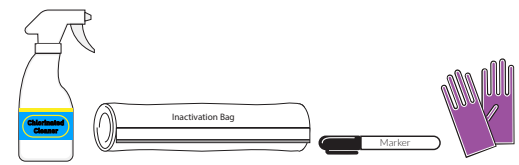
Experiment Protocol

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.*

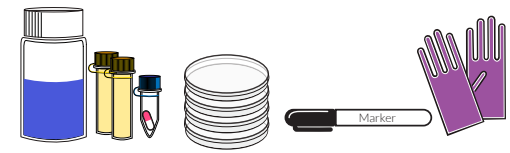
Student's Experiment Protocol

1. Creating SC Agar Plates Day 1, 25 minutes

Goal Create non-selective and selective SC agar plates.

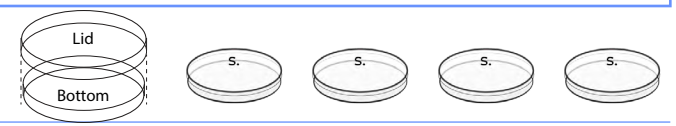
Materials from your kit

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



Prepare

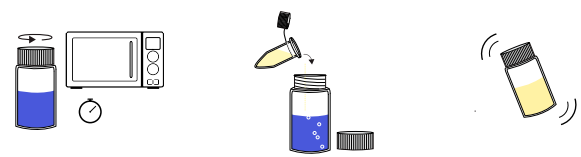
1.1 Label the bottom of the petri dishes with a marker with your initials or your team/group name (*the bottom is the part that has the smaller diameter of the two: the bottom fits inside the lid).*



Mix the Agar

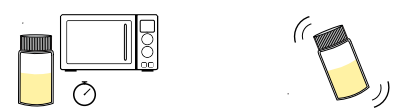
1.2 Unscrew the lid from the sterile water bottle and keep it loosely on top of the bottle to prevent any contaminants from entering the water, but allowing air to escape. This will prevent pressure build-ups.

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.

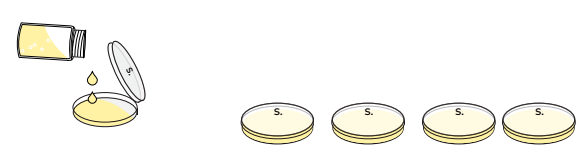


1.6 Repeat this step one more time, then swirl to mix for 30 seconds or until you see all powder dissolved.

If you are familiar with LB agar petri dishes for bacteria, you'll notice this agar is a clear white color instead of the yellow color of LB agar. That is because bacteria and yeast need different nutrients and those give different shades to the agar mixtures! Also notice you don't need antibiotic to make the agar selective. SC agar is already selective agar; go to page 12 to learn why.

Pour your selective agar petri dishes

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. If the agar does not cover all the bottom, gently tilt it.

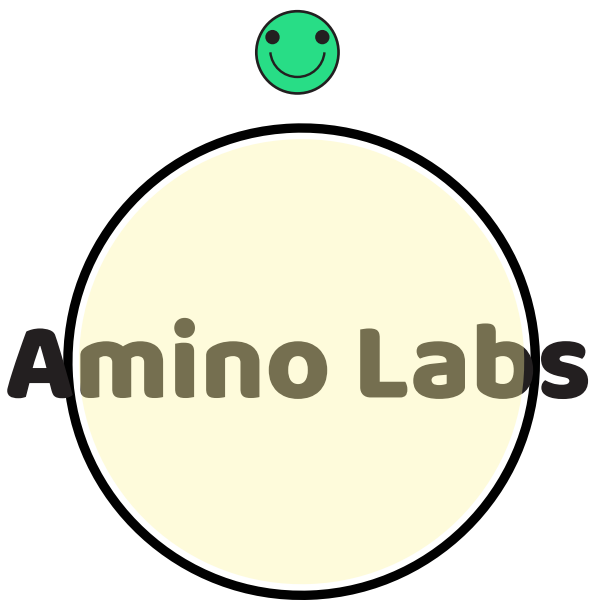


1.8 Place the lid ¾ of the way back on to let the agar can cool and dry (solidify).

1.9 Let the agar harden. You will use one of the petri dishes in the next step. Once they are solid, put the remaining 3 petri dishes in their original ziploc bag for later use, and store in a refrigerator. Depending on your climate, it can take 5 to 30+ minutes for the agar to solidfy.

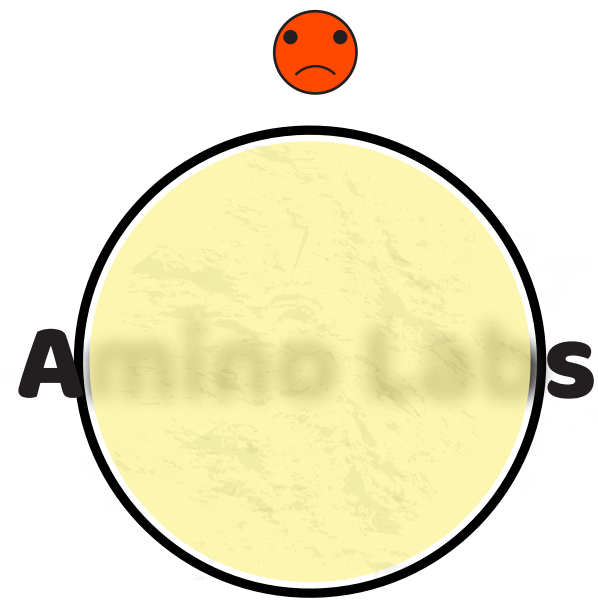
Checkpoint - Agar Plates

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.

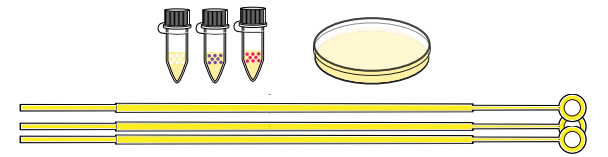
Unfortunately, if the agar does not solidify, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

2. Create your painting palette Day 1, 25-45 minutes + 24-48 hours wait time

Goal **Create yeast paint painting palettes.**

Materials from your kit
(1) Selective agar plates
(3) Yellow loops

Tubes of yeast paint

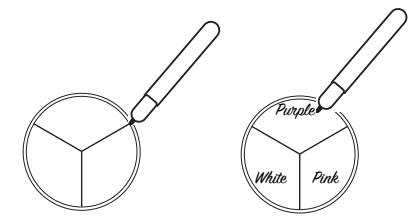


Prepare

2.1 If you have an incubator, turn it on to 30°C.

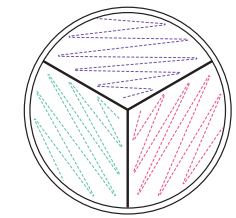
Streak

2.2 Using a sharpie/permanent-marker, divide up the bottom of the petri dish you kept to use into 3 sections, the same way you would divide a pie or cake.



2.3 Use the sharpie to write the name or the abbreviation of the different colors of yeast in each section on the bottom of the petri dish. One color per section. The order does not matter, as long as each color has a section.

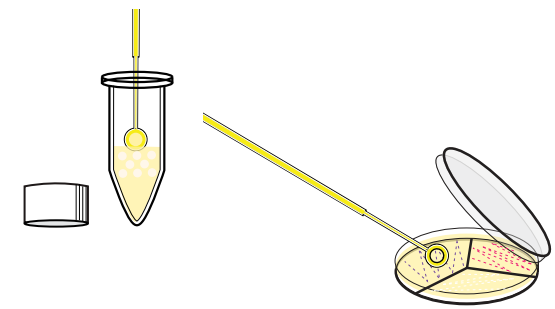
2.4 If you have printed instructions, you can place your petri dish on top of the stencils on the next page. Your goal will be to use a yellow loop that you will dip in your yeast before you trace the zig zag pattern. If you are using online instructions, you can manually copy the right stencil pattern on the back of one of your blank stencil and place your petri dish on top, or you can even freehand it - have a look at the zig zag pattern you are trying to reproduce. For this streaking, it is not important to be precise, it is simply necessary to cover most of the surface area with the yeast.



2.5 Open one of the yellow loop by holding the straight end of it, not the loop end. Remove from the packaging. Don't let the loop end touch anything yet!

2.6 Open one of your colored yeast tube. Dip the loop end of the yellow loop into the stab of colored yeast. The texture will be that of soft jelly. If any jelly gets stuck on the loop, that's ok! You can go to the next step.

2.7 On your petri dish, find the section that you marked for this color and, using the end of the loop you dipped in the colored yeast, trace the zigzag line like on the stencil on the next page.

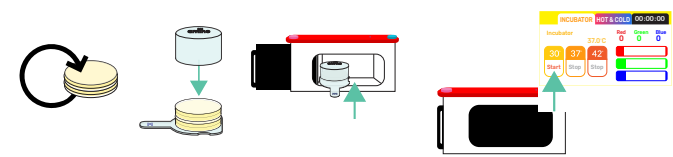


2.8 Discard the loop in your inactivation bag or discard container.

2.9 Using a new yellow loop each time, repeat steps 2.5 - 2.8 for each colored yeast tube you have.

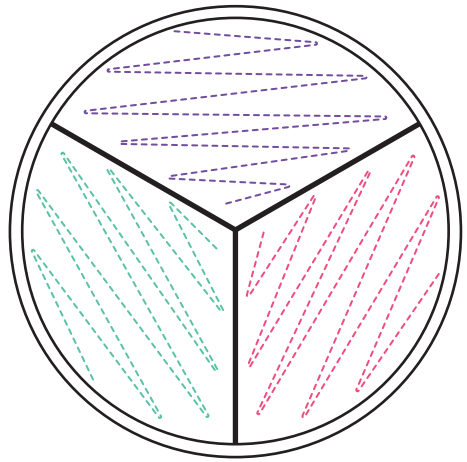
Incubate for 24-48 hours

2.10 Incubate your streaked plate **upside down** at ~30°C for 24 to 48 hours. This will be your painting palette of yeast paint to create your living art. (It may take longer to incubate if you do not have an incubator, up to 5 days. Note that the colors will be more pastel if you are incubating at room temperature).



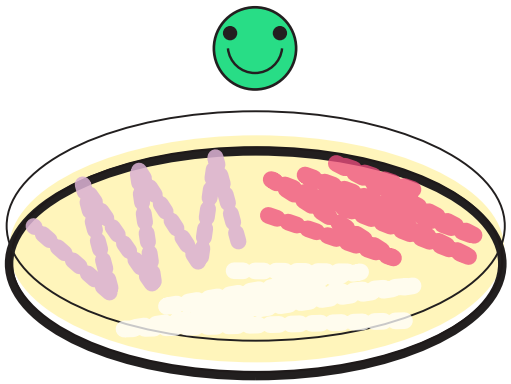
Note: Remember to flip your plate upside down! If you have Amino Labs' DNA Playground, remember to put the humidity chamber on top of your plate and to close and lock the incubator door.

Stencil

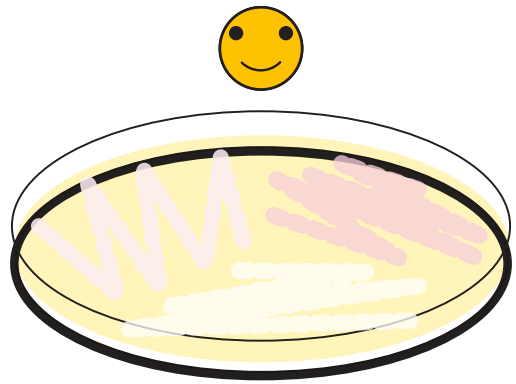


Checkpoint - Yeast Paint

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

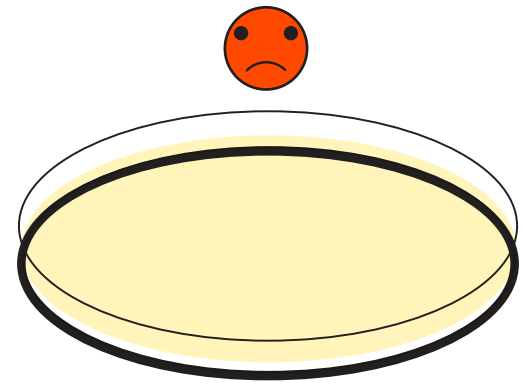


A perfect yeast plate has lots of brightly colored yeast after incubation. Proceed to the next page. This suggests that you have made your SC agar petri dishes properly and have incubated long enough at the right 30°C temperature. Note that the pink bacteria grows a bit slower than purple and white. That's normal! As long as you have some pink bacteria, you are good to go. Congratulations!
If you are incubating at room temperature, your yeast will not become as bright as below. That's ok!



A yeast plate showing light colored yeast requires more time to grow. Continue incubating, checking every 12hrs, until the colors are bright.

If you have to save time you can paint your art once you start to see the yeast color on your painting palette. You won't get to see what the colors will look once bright before you create your art, but your art pieces will still color brightly if you incubate them long enough.



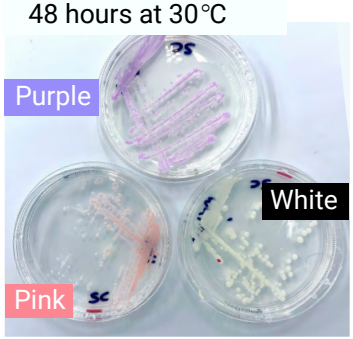
If you see no growth on your plate:

1. If your incubator was not at 30°C or is home-made, 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 on the same petri dish.
3. If you still have no colonies after repeating Step 2, contact us at help@amino.bio. We will help you succeed.

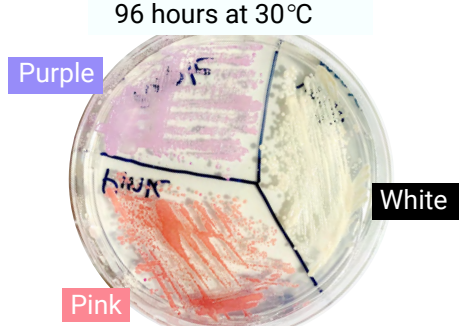
What can you expect the colors to look like?

Under regular light

48 hours at 30°C



96 hours at 30°C

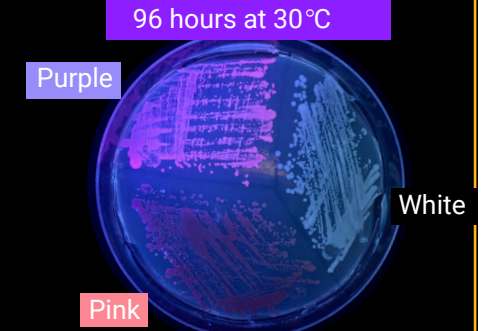


Under blacklight

Notice that the purple yeast-fluoresces pink under blacklight, while the pink yeast does not fluoresce!

48 hours at 30°C

96 hours at 30°C

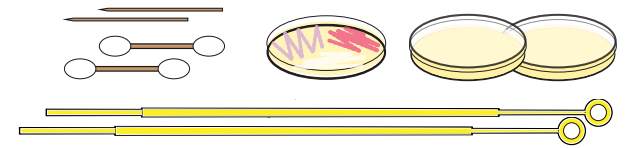


3. Paint with yeast! Day 2, 30-60 minutes + 24+ hours wait time

Goal **Create living paintings**

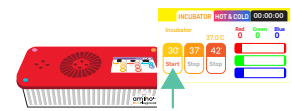
Materials from your kit

Selective Agar petri dish
Painting palette
Loops
"Yeast Paintbrushes"



Prepare

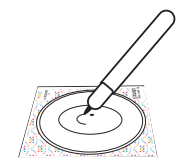
3.1 Make sure you have colored yeast on your biopaint petri dish from the prior day. If colors have not appeared yet, wait longer, up to 48 hours. Once you have colors, take your painting paletter petri dish from the incubator.

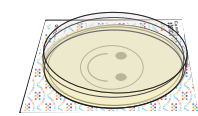


3.2 If you have an incubator, turn it on to 30°C.

Paint!

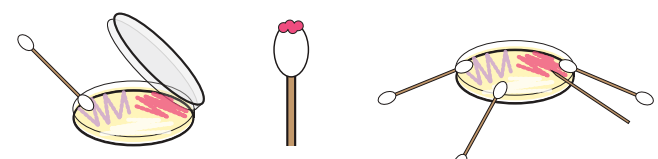
3.3 Using the blank stencils in your kit, sketch your art piece for each petri dish your are painting. You can also use the canvas stencil that already has an image included in the kit.





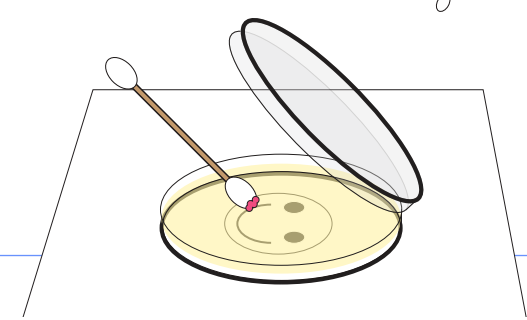
3.4 Set one of your selective petri dish canvas on top of your sketched stencil or the image stencil from the kit.

3.5 Using the yeast "paintbrushes" (the sterile cotton swabs and toothpicks) and your remaining loop, paint your art onto the agar by dipping into the colored yeast from the biopaint petri dish and tracing your image, gliding on top of the agar. The agar is like a Jell-O, be careful not to puncture it as you paint.



Note: Assign a yeast color to each paintbrush as you only have a few of these. Set them down on the edge of the yeast painting palette when you are not using them until you have completed your art. You will not see the yeast appear right away, but you may be able to see a “wet” trace where you have painted on top of the agar. **You only need to dip into the colored yeast on the painting palette once to collect paint.**

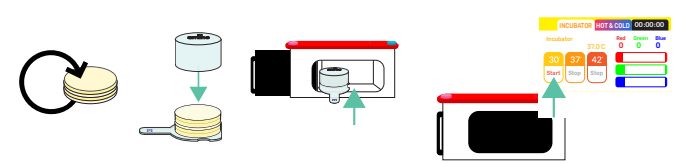
3.6 You do not need to paint all canvases at the same time. In fact, we recommend painting 1 or 2 the first day and seeing how the image develops while it incubates. Yeast painting is an art that surprises!



Return any unused petri dish canvas, the painting palette petri dish and the paintbrushes to the ziploc bag and refrigerate.

Incubate

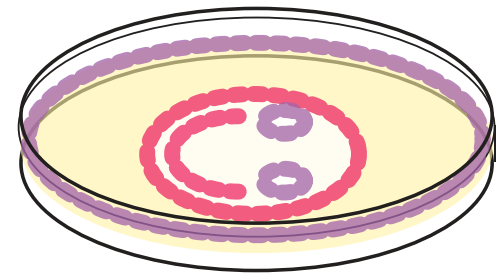
3.7 Incubate your art **upside down** at ~30°C for 24 to 72 hours (it may take longer if you do not have an incubator). You must flip your plates upside down so that the agar is up and the lid down. Place it on top of the incubator paddle if you have Amino Labs’ DNA Playground, and place the incubator humidity chamber on top before sliding it into your incubator.



Note that you can only incubate 2 petri dish canvases at a time in a DNA Playground Home size. If you painted multiple canvases, place them in a ziploc bag with your painting palette and refrigerate until you are ready to incubate them.

4. Did your living art grow? Day3+

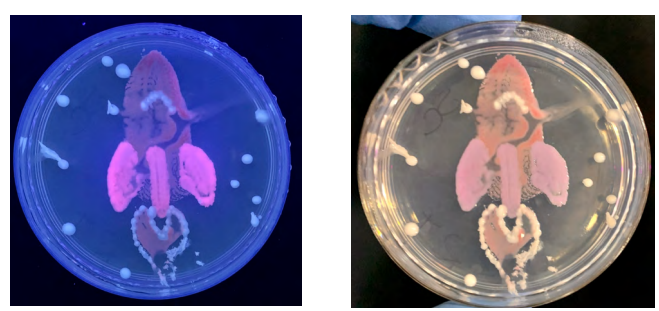
Goal **Verify if your yeast paintings grew**



You should see your living painting appear over the next 24 to 96 hours! Keep an eye out, and your camera ready to document. Congratulations!

If there are any unused petri dishes left you can repeat the painting steps for those canvases or keep them in a bag in the reefrigerator for up to a month. If you see any unexpected growth on these like fuzzy, dark spots, follow the inactivation instructions.

Note:
If you cannot see any growing cells at all after 48 hours of incubation at 30C, your experiment may have failed. See our troubleshooting guide at the end of the manual, compare results with your group, if applicable, or contact us with photos of your result and any documentation of your process so that we can help you succeed in the future. Make sure, if possible, to also review the video tutorials on the youtube channel (youtube.com/c/aminolabs) to see if you missed any steps!



Rocket by Amino Labs @aminobiolab

CONGRATULATIONS

You have now joined the global community of bioartists! Happy with your artwork? There are many opportunities to share it online, exhibit it in your community and even participates in contests and artist communities on the web!

Share your results with friends and our growing community. Find us on Instagram, Twitter, and Facebook @ aminobiolab

Don't forget, you can preserve your bioart with our *Keep-it Kit™* . 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.

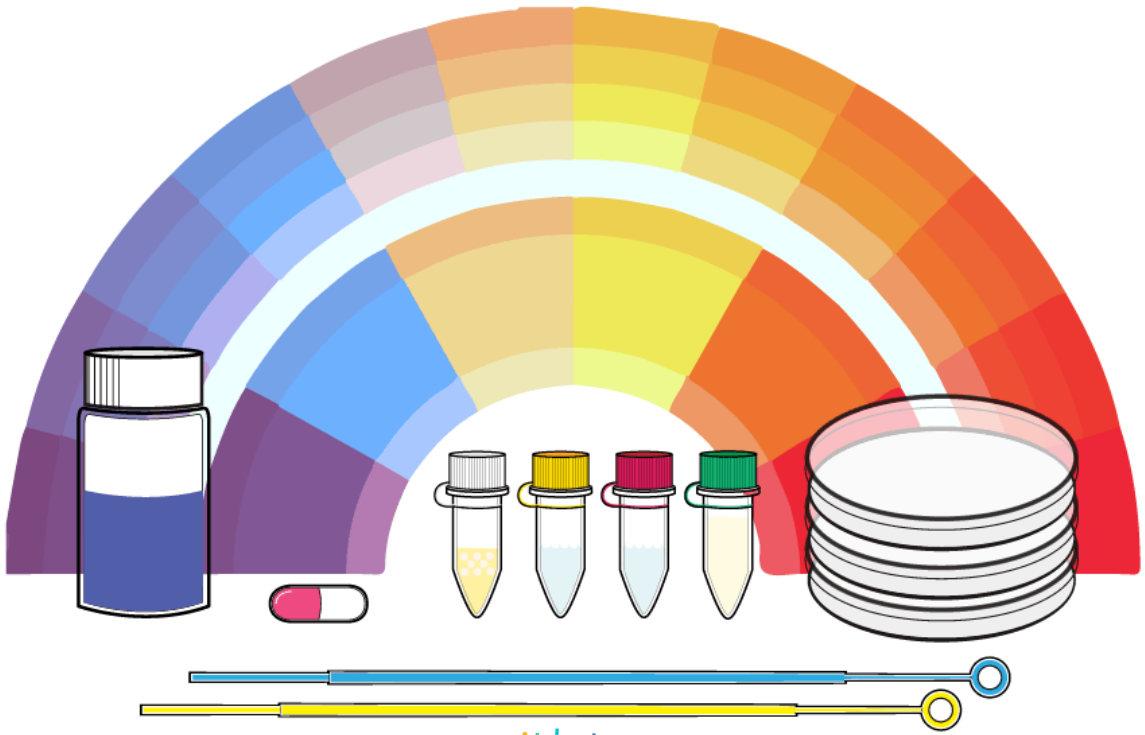
If anything else causes you issues, please contact us : help@amino.bio

More Information

Made in Canada

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

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



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