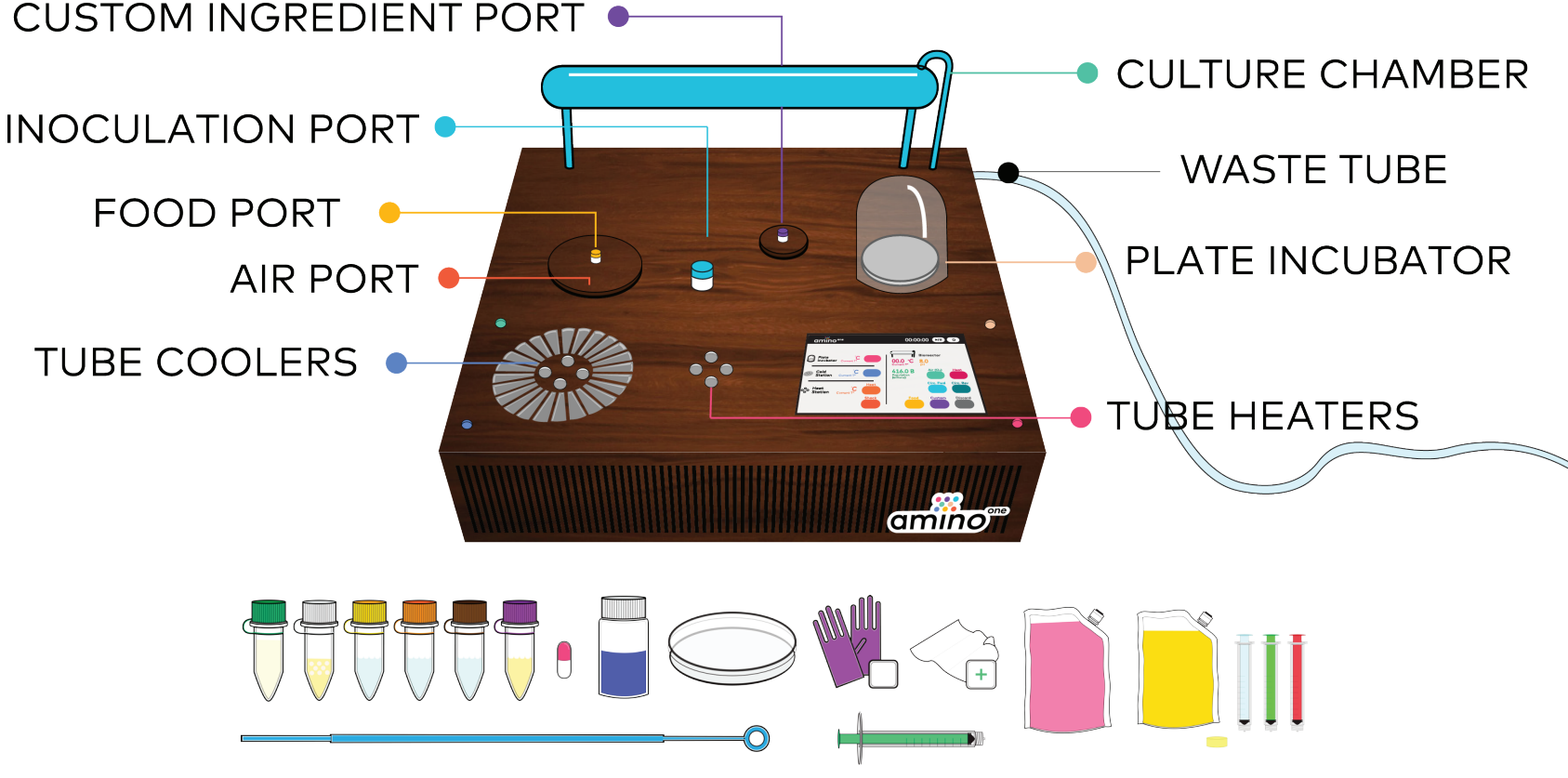
Bioengineering with the

amino one alpha



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General safety rules

Bioengineering is a safe activity when you follow the rules.

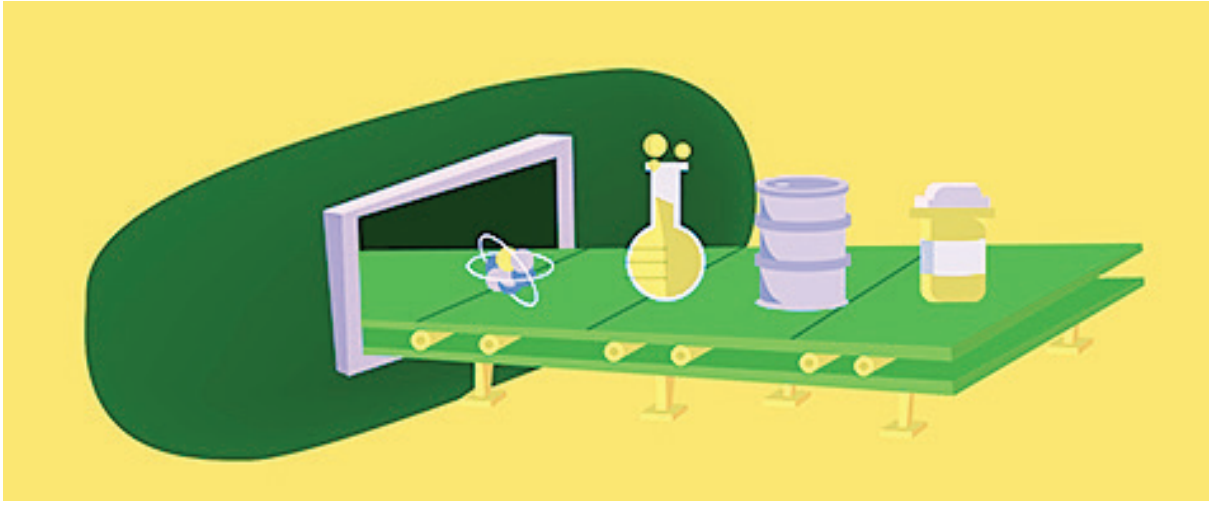
The kit in your hands contains **only non-pathogenic ingredients.** These are rated Biosafety Level 1, the lowest level (and therefore the safest). With the ingredients, no special containement or training is required in North America*, but you must follow the following rules for your safety and the success of your experiment(s).

We recommend the system for ages 8+, under adult supervision. We recommend that the waste container be emptied by an adult and that the cleaning instructions be stricly followed for safety and experiement success. Make sure to store the ingredients in accordance with the instructions found in this booklet. Eyewear is not provided but can be worn if desired.

*If you would like to do a short online lab safety course for your edification, we recommend this goverment of Canada course: training-formation.phac-aspc.gc.ca

- **Do not eat or drink near your experiments.** Keep your experiment at least 10 feet from food, drinks, etc.
- **Wash your hands before and after** manipulating your experiment, the ingredients, or the hardware.
- **Wear gloves**, especially when cleaning your Amino One. Any latex, nytrile, or general purpose gloves you can find at the pharmacy will do.
- **Place the Amino One on a stable worksurface.** Keep it level at all times.
- **Make sure there is no kink, folds, or blockage in the waste tube**. The pressure build-up caused by a kink in the tube could break the Amino One and create leaks.

What is biological engineering?



Bacteria are beautiful and fascinating—and they're also, part of a revolution in sustainable advancements across food science, energy, health, and materials. Thanks to the hard work of scientists around the world, we can now program bacterial DNA to produce things for us, just like mini factories!

Usually when we think about bacteria it's because we're trying to get rid of it, but a growing number of scientists, engineers, and hobbyists are using it as a stepping stone for tomorrow's innovations in all spheres of life. From the food you eat, the medecine you take, to materials you use—programming DNA is improving our quality of life.

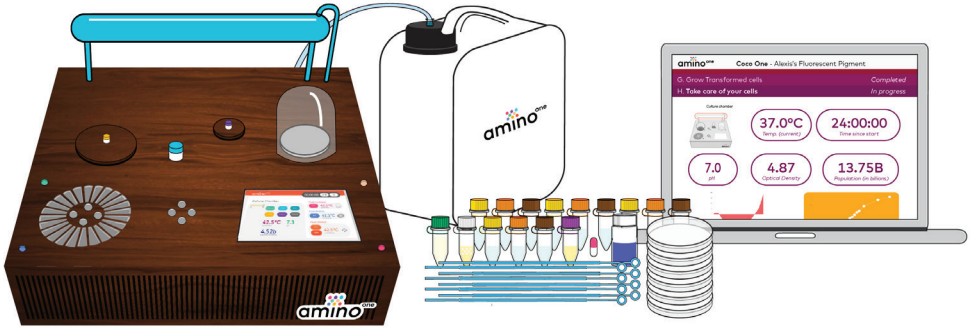
By getting hands-on experience with genetic engineering and biotechnology, you will become immersed in some of the most cutting-edge science of the 21st century.

The Amino One Ingredient Kits have everything you need to bioengineer for the first time or the hundreth!

In this booklet, you will learn how to:

- Set up and use your Amino One unit
- Connect the wifi for data analysis / sharing
- Store bioengineering ingredients
- Run experiments

How does the ecosystem work?



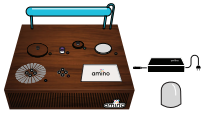
The Amino One is an ecosystem for engineering with biology. Learning and prototyping with genetic engineering and cells is starting to become accessible to newcomers of all ages and backgrounds.

We hope these explanations are easy-to-follow, but they may contain some new terms. For your reference, we have included a glossary at the end.

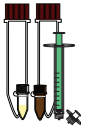
Amino One's Ingredients Kits make it easy to add a DNA program into living cells. Once you add the program, you can then grow and take care of those cells so that they can produce something for you—light, pigment, flavours, materials, and more!

Each ingredient in the kit is pre-measured and labled for ease-of-use. The Ingredients Kits can be combined into a themed *App*, For example, the Ingredients Kits in the Artist App allow you to take a colour-generating DNA program copied from corals and insert it into bacteria. This App can include the *Extract-it kit* which allows you to extract the pigments grown in the bacteria so you can paint or draw!

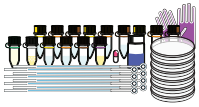
Amino One Ingredients Kits



Hardware: The Amino One Desktop Biolab is a culturing and DNA transformation tool that enables you to perform complex tasks on a simple, self-contained platform.

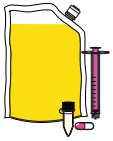


Extract-it Kit: For an end-to-end bioengineering experience, Amino One provides you with the tools to extract and purify what your bacteria produced so you can actually use it. **Requires access to a centrifuge*



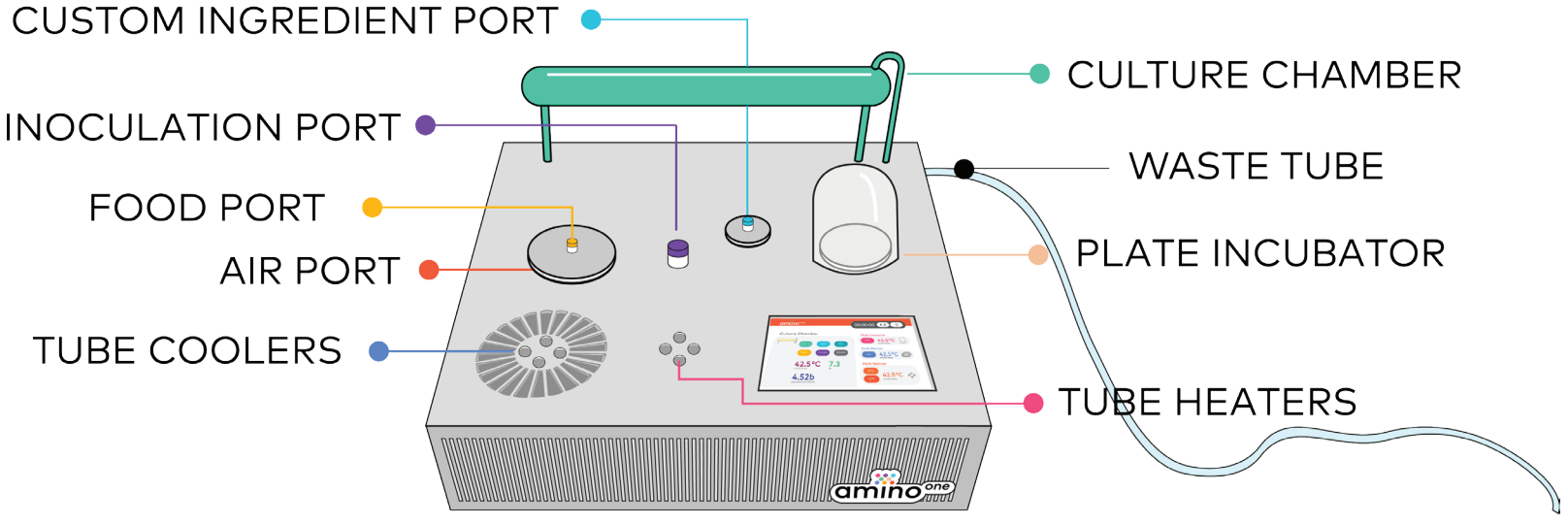
Engineer-it Kit: Insert a DNAplasmid into living cells using Amino One. The plasmids are themed according to the Experience.

Clean-it Kit: Like all other lab equipment, the Amino One needs to be kept sterile both before and after your growth experiment.



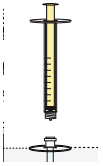
Grow-it Kit: Keep tabs on how your cells are growing and take care of them so that they can multiply and execute their new DNA program.

Amino One Desktop Biolab



Description of parts

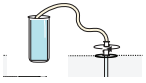
Culture Chamber The chamber has temperature control, aeration, and sensors to help you take care of your bacteria as it multiplies. When bacteria are grown in a liquid, this is called a culture,, bacterial culture, or growth.



Inoculation Port This Port allows you to inject the transformed bacteria into the culture chamber. It connects to a syringe which you will use to insert the bacteria in order to prevent leaks and contamination.

Food Port and Connector Supply your bacteria with food through this port by connecting the sterile food containers with the food connector provided. Keep this connector sterile by placing it in boilling water 5 minutes before and after use. Store in a clean bag.

Plate Incubator To grow optimally, bacteria are kept in incubators, which are contained environments with regulated temperature. The safe strain of bacteria included in your Ingredients Kits prefer a temperature of 37˚C.



Custon Ingredients Port This port allows you to inject a custom liquid or balancing solution into your culture to experiment.

Tube Heaters / Heat Station To bioengineer, you need to use heat for several things: 1) to keep ingredients warm; 2) to heatshock bacteria to allow the DNA program to go through the cell membrane; and 3) to recover them. Amino One has 4 tube heaters that can be set at 37˚C for incubation / ingredients warming, and 42˚C for heatshocking.

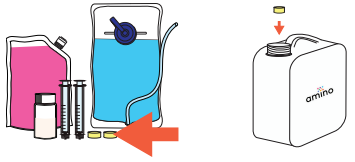
Tube coolers / Cold Station To transform bacteria you need to both keep certain ingredients “on ice” and to “ice” bacteria. We’ve replace the need for ice in Amino One with tube coolers that reach a freezing temperature of 1-6˚C.

Waste Tube As you fill the system with food and run new experiments, extra liquid will be collected in a waste container that contains an inactivation tablet.v

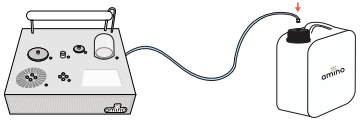
Setting up your system : continuous culturing

Your Amino One is a continuous bioreactor, which means that waste exits the system periodically. A waste container with inactivation solution is connected to collect this overflow. You may have an Amino One waste container. If not, a 2L /4L bottle will do.

Make sure to securely insert the waste tube and allow for air venting, as air from the system will be channeled there. We suggest driiling holes in the lid of your bottle—one for the tube, one for the

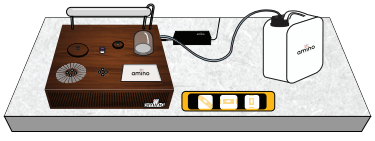


1. Using gloves, drop an inactivation tablet from the Clean-it Kit inside the waste container. This will kill any bacteria as they enter the waste system.



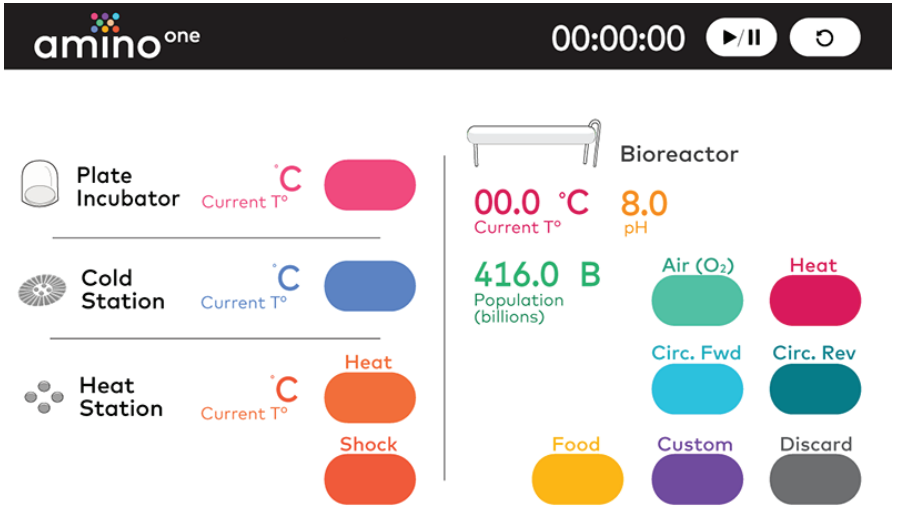
2. Connect the waste tube to the connector on the lid of the waste container. If you have your own container, make sure it is a min of 4L capacity. ***Make sure there are no kinks in the hose!***

3. For other wastes from your experiment like tubes and inoculating loops, get a used yogurt container or similar and discard your items within. The *After your experiment* section explains how to safely dispose of it all after your experiment.



4. Verify Amino One is level and stable. And you are ready!

Setting up your system: screen controls, sensor data



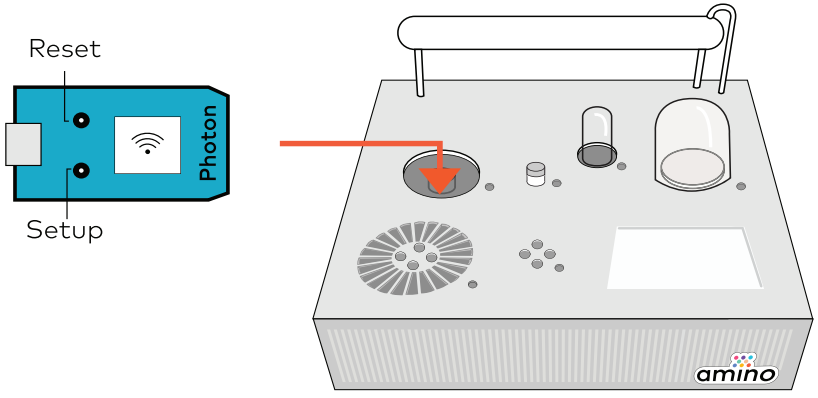
Amino One has a touchscreen that allows you to control the different system parts and let you know what's going on inside—you will see the temperature, number of grown cells (in billions!), and the pH level. These data points will help you take care of your bacteria and experiement further... What happens if you lower the pH? Feed your bacteria pure sugar? You will be able to see the results in real time!

Above is the screen layout you will find on your machine. In the largest block on the left, the first row of buttons refers to the circulation mechanism inside the culture chamber—it allows you to heat up the liquid to 37˚C, and to change the flow direction FWD / REV.

The second row allows you to turn on the air flow in the reactor (AIR (O2)), activate the different ports to uptake solutions (Food, Custom Ingredients), or to empty out the machine with Discard.

The smaller blocks on the right activate the temperature stations—the Plate Incubator which reaches 37˚C, the Tube Heaters which reach 37˚C, and 42˚C and the Tube Coolers which reach 1-6˚C. Press each button to activate it, and press it again to turn it off.

Setting up your system: connect to WiFi, visit your WebApp



This sensor data can also be sent to your own Amino One WebApp via a wiFi module in the machine. This allows you to monitor your experiment from afar every step of the way, and share the results of your work with your friends and family.

The WebApp is in early Alpha stage and will be rolled out throughout the fall 2016. Check the google group for updates & info!

1. To connect, first download the "Particle by Particle Industries App" on your mobile device (search the App Store or the Google Play store).

Follow the instructions in the app. You will need to create an account and press the small Reset button on Amino One located on the inside of the food port.

2. To see your Amino's WebApp, find your Amino ID engraved on the back of your machine, and visit:

www.amino.bio/aminoone/**aminoID**

Technical Specifications

General
Dimensions: 14 x 10 x 8 in
Weight: 3.6 Kg
Chamber volume: 150 mL

Power
Voltage: 110-250
Frequency: 50-60 Hz
Average power: 40 W
Peak power: 100 W

Operating environment
Temperature: 16 - 25 ˚C
Humidity: 20 - 90 %
Altitude: 3,000 m

Connectivity
Network wifi: 802.11n
Screen: 5.0 inch, capacitive

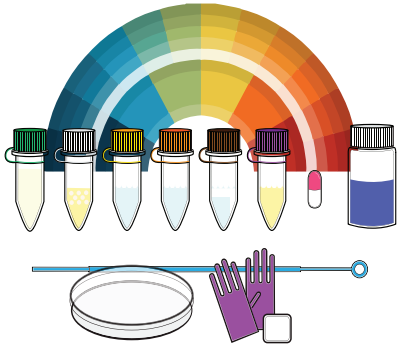
Culturing
Chamber: Tempered Glass
Population: 0 - 1000 B cells
Temperature: 37 C +/- 0.5 ˚C
Filtered aeration: Yes

Temperature Stations
Tube Coolers: 1 - 6 ˚C
Tube Heaters: 37/42 ˚C
Plate incubator: 37 ˚C

Realtime Sensor Accuracy
Temperature sensors (4) :
Bioreactor +/- 0.1 C @ 37 ˚C
Tube Coolers +/- 0.1 C @ 1˚C
Tube Heaters +/- 0.1 C @ 42˚C
Plate incubator +/- 0.1 C @ 37 ˚C

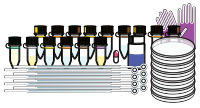
pH: 0-14 +/- 0.1 @ 37 ˚C
O.D. (~600 nm): +/- 1 nm @ 600 nm

Ingredients Kits

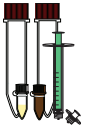


The Different kits that make up an Experiment

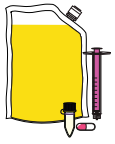
* Not all Experiment include the Extract-it Kit



Engineer-it Kit: Insert a DNA program plasmid into living cells using Amino One. The plasmids are themed according to Experiment



Extract-it Kit: For an end-to-end bioengineering experience, Amino One provides you with the tools to extract and purify what your bacteria produced so you can use it outside of the system.



Grow-it Kit: Keep tabs on how your cells are growing and take care of them so that they can multiply and execute their new DNA program.

Clean-it Kit: Like all other lab equipment, the Amino One needs to be kept sterile both before and after your growth experiment.

Technical Specifications

Engineer-it Kit
For Bacterial Transformation

Growth plates: 6 cm petri dishes (3)
DNA plasmid 250 ng (1)
Antibiotic: variable
Transformation Buffer: 75 uL tubes (2)
Recovery media: 350 uL tubes (2)
Cells: K12 E. coli Stab (1)
Solid growth media:
LB agar powder (2 g)
50 mL sterile water

Grow-it Kit
Liquid Bacterial Culturing

Liquid Growth media: Sterile LB media (200 mL)
Antibiotic: variable

Extract-it Kit :
Protein Extraction & Purification

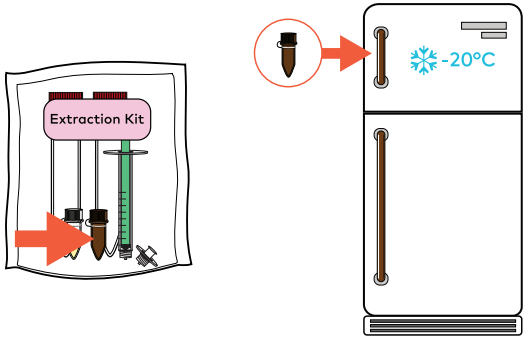
Lysis buffer: 1 mL
Lysis Accelerator: 5 mg
Filtration: (1) 0.22 um filter
Tubes: (2) 15 mL & (1) 1.5 mL Screw Cap
Syringe: (1) 3 mL

Clean-it Kit
Sterilisation & Inactivation

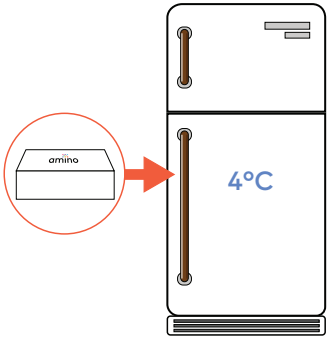
Inactivation Solution A: (1) 500 mL
Inactivation Solution B: (1) 20 mL
Syringe: (3) 10 mL

Unpacking and storing your ingredients

Follow these instructions to give your ingredients a better shelf life and promote successful experiments:



1. If you've ordered an **Extract-it** Kit, take the brown tube out of the package (Lysis Accelerator) and place it in a freezer.



2. Place the rest of the ingredients in a standard refrigerator at around 4˚C.

Other supplies you may need

Depending on which Kit you will use, you may need some of these items. Make sure you have them on hand before starting.

- 2L of Distilled water (cost prohibitive to ship, but you will be able to get it cheaply from a grocers or pharmacy. Tip :Pick up a 4L jug that you can use for waste containers!) **(Clean-it Kit)**
- Access to a microwave or hot plate/stove **(Engineer-it Kit)**
- A disposable container for discarding used tubes, etc. (e.g. yogurt container, plastic soda cup) **(All Kits)**
- Latex, nytrile, or similar gloves **(All Kits)**
- Bleach or Isopropyl alcohol (rubbing alcohol) to clean up your work surface(s) **(All Kits)** Note: You may use leftover Clean-it Kit "Solution A" to wipe down surfaces

Example timeline for an App with the *Extract-it Kit*.

Create petri dishes, LB agar and streak blank bacteria
(60 minutes)

monday
1:00 pm

24 hours

Make cells chemically competent & transform with DNA plasmid
(120 minutes)

tuesday
1:00 pm

CAN STOP AND STORE UP TO 1 WEEK

24 hours

Run clean cycle & pick a colony and inoculate the main reactor
(60 minutes)

wednesday
1:00 pm

24 hours

Collect bacteria, Lyse bacteria and clean the Amino One
(120 minutes)

thursday*
1:00 pm

CAN STOP AND STORE UP TO 1 WEEK

24 hours

Collect and sterilize the pigment (Amino One is not required)
(30 minutes)

friday*
1:00 pm

*Centrifuge requires

Example timeline for an App without the *Extract-it Kit*.

Experiment protocols

Note to User

The Amino One Experiements enable users to learn the basics of genetic engineering using an Amino One Desktop Biolab. An Experiement consists of several kits allowing the user to complete the following hands-on exercises:

Engineer-it Kit: Do a bacterial transformation

1. Make selective and non-selective plates for growing bacteria
2. Grow/streak K12 *E. coli* (these are used for DNA transformation)
3. Make K12 *E. coli* cells chemically competent (able to take up DNA plasmids)
4. Transform the competent cells with DNA programs for generating colour pigments
5. Recover and grow the transformed cells on plates

Grow-it Kit: Culture bacteria

6. Load an Amino One with growth media + antibiotics
7. Pick a colony and culture the transformed cells in the Amino One reactor
8. Monitor growth and inject other liquid into the culture

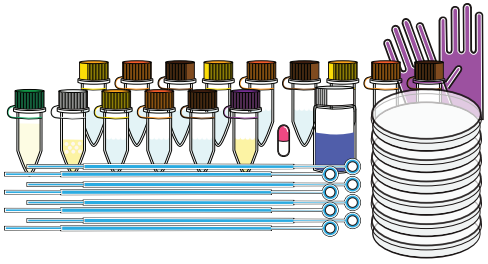
Clean-it Kit: Sterilize the Amino One Desktop Biolab

9. Inactivate the bacterial culture and sterilize the Amino One

Extract-it Kit: Use the products
Note: not all apps require/use the Extract-it Kit

10. Collect the cells after the culture has grown and the pigments have been expressed
11. Lyse the cells (break them open), and release the pigments
12. Collect, filter, and sterilize the pigments — then paint with them!

Engineer-it Kit Overview



Cells are tiny, living units that function like mini-factories. Bacteria are single-celled organisms. Individual bacteria can only be seen with a microscope, but they reproduce so rapidly that they often form colonies that we can see. Bacteria reproduce when one cell splits into two cells through a process called binary fission. Fission occurs rapidly, in as little as 20 minutes. Under perfect conditions a single bacterium could grow into over one billion bacteria in only 10 hours!

Each bacteria, or cell, 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 tells the cell(s) how to function.

In this kit, you get a DNA plasmid. DNA plasmids are also a set of instructions for the cells, but much smaller—a plasmid only has a few functions, as opposed to the complete genome of the bacteria. Bacteria share vital information with each other by passing along plasmids. By inserting a new plasmid in our bacteria, we can get them to produce things for us. In the case of the Artist App, the plasmid encodes for the creation of pigments.

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

The bacteria you will find in your kit is standard lab bacteria commonly used to bioengineer, *E. coli* K-12.

You will need to grow your bacteria on the nutrient agar petri dish (plates) before you insert the DNA plasmid. Freshly grown bacteria take up DNA much better than older ones since they are still in a growth phase. Nutrient agar is a jello-like staple food source for the bacteria which you will pour into the provided plates (petri dish) in the first step. Two types of agar will be made: non-selective and selective. The non-selective agar allows any bacteria to grow, while the selective agar has an antibiotic mixed in which allows only antibiotic-resistant bacteria to grow. The DNA plasmid you insert will make your bacteria antibiotic-resistant so that only those cells will grow.

Steps to Engineer Cells

1. Make selective and non-selective plates
   Day 1, 20-45 minutes

2. Grow/streak K12 E. coli (used for Transformation)
   Day 1, 20-45 minutes + 12-16 hours incubation

3. Make K12 E. coli cells chemically competent
   Day 2, 10-25 minutes

4. Transform the competent cells with DNA plasmids
   Day 2, 90 minutes

5. Recover, grow the transformed cells on plates
   Day 2, 10-15 minutes + overnight incubation

Each Engineer-it kit has enough ingredients for **up to 2 users or teams**. If you are alone, you can do 2 transformations simultaneously, or keep the extra to use another time.

Kit Components

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. [1]

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

Agar Powder : This LB agar powder is industry standard and comes in 1g increments. Each gram of LB agar powder can make 25 mL of molten LB agar (4% w/v). [1]

Cells: This standard K12 strain of E. coli is non-pathogenic and is the prototypical strain used by thousands of labs around the world. This strain comes as a "stab", a small tube which contains agar . [1]

DNA: A DNA plasmid to program your bacteria.

Antibiotics for Transformation: Amino Labs' proprietary antibiotic delivery system helps stabilize antibiotics for shipping and long-term storage. These capsules have a measured amount of antibiotics for 45 mL of molten LB agar. In such small quantities, these antibiotics are very safe, even if ingested by accident. Try not to ingest them, however! [1]

Sterile Water : Sterility is critical when you're doing biotechnology. This is distilled water that has been sterilized with an autoclave in order to ensure there are no contaminating organisms present. This 50 mL volume, when used with 2 g of LB agar powder is enough to make 5 LB agar plates. [1]

Small Loops: Small inoculating loops are used for transferring 1 uL of liquid and/or other tasks. These replace costly traditional pipets. Package of 8.

Large Loops: Large inoculating loops are used for transferring 10 uL of liquid and/or other tasks. Yellow loops are great for spreading out bacteria after a transformation. Package of 8.

Petri Dish / Plate: 6cm petri dishes are large enough for typical lab experiments and help save on cost of reagents as well as reduce waste. This is a package of 5 sterile petri dishes.

[1] For education purposes only.

1. Creating LB Agar Plates

20-25 minutes

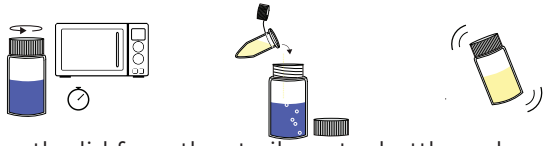
Goal

Create non-selective and selective LB agar plates.

Materials from your kit

(1) 50 mL sterile water
(2) 1 g LB agar powder
(1) antibiotic pill
(3) 6 cm petri dishes

1.1 Label the bottom of the 6 cm petri dishes
1x: Non-selective [your name] **2x** [Antibiotic Name] [your name]



1.2 Remove the lid from the sterile water bottle and gently set it on top of the bottle

1.3 Place the bottle in the microwave for ~30 seconds (until you see it boil). Don't let it boil too long or you will lose water due to evaporation. **Careful, the bottle will be hot!**

1.4 Add both tubes of LB agar powder. Careful, the water may boil as the powder is added.

1.5 Microwave for another 5 seconds. Swirl until the powder is fully dissolved. If the solution is still cloudy, microwave another 5s. Careful, the liquid can boil over.

Make non-selective plate

1.6 Pour molten LB agar in your non-selective labelled petri dish. Just enough to fill the bottom. Swirl the plate to make sure the molten LB agar fills the bottom. Place the lid back on top.

Make selective plates

1.7 Let the bottle cool to ~65˚C by letting it stand for ~2 minutes.

1.8 Once the molten LB agar has cooled to a point where it is just bearable to the touch (should still be hot), add the antibiotic pill and gently swirl for a few minutes until the contents have dissolved. Do not introduce bubbles into the LB agar. Don't swirl too vigorously. The gelatin capsule may not fully dissolve, this is acceptable.

1.9 Once the antibiotic pill is dissolved (or the contents of the pill has dissolved), pour the molten LB agar into the remaining petri dishes. There should be enough for the 4 remaining plates. Even if you are doing 1-3 transformation, pour all the plates now.

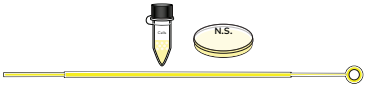
1.10 Let the LB agar harden. The non-selective plate is used in the next step. Put the remaining selective plates in their original bag for later use and store in a refrigerator.

2. Growing Blank Cells

20-45 minutes + 12-16 hrs wait

Goal

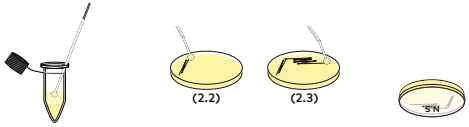
Streak plates with blank cells for fast-growing, well-separated colonies to be engineered



Materials from your kit

(1) Non selective plate
(2) Yellow Inoculation
(1) LB agar stab of K12 E. coli

2.1 Turn on the Amino One **37 ˚C plate incubator**



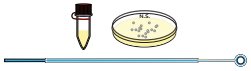
2.2 Open the loop bag on the handle side to avoid contamination and, taking one yellow loop, dip it in the stab of cells, streak it in a small zig zag on the non-selective plate.

2.3 Using a second clean inoculation loop, make a single pass through the first zig zag and continue to zig zag over the rest of the plate.

2.4 Incubate your cells on the non-selective plate at 37˚C for 12-16 hours with the lid down. *It is important to do the following steps in 16 hours or less because this is when cells are growing fast and take up DNA best!* Your cells will grow overnight and be ready for the transformation tomorrow.

3. Making Chemically Competent Cells 10-15 minutes

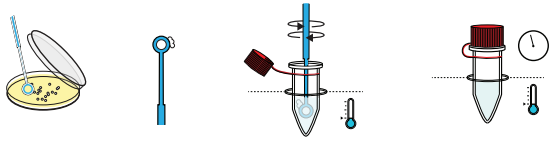
Goal
Pick colonies and add them to cold transformation buffer, making them able to take up DNA (or in scientific terms, "competent").



Materials from your kit

(1) Streaked non-selective plate with K12 *E. coli*
(1-2) Blue loop (1 uL)
(1-2) Transformation buffer

3.1 Turn on the Amino One **Tube Coolers (~4˚C)**



3.2 Put the T. Buffer tube in the tube cooler (1 per user/team or transformation. You can do up to 4.)

3.3 Using a blue inoculating loop, scrape ~10 small separated colonies on the tip of the loop. You should be able to see some white on the end of the loop.

3.4 Immerse the loop in the cold Transformation Buffer without touching the sides of the tubes. Twist and stir the loop to mix the cells in the buffer.

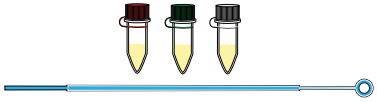
3.5 Twist the loop like a blender to suspend the cells. There should be no clumps floating in solution. You may have to do this vigorously. If you see clumps, keep blending. Note that this should be done at 4˚C; Keep the tubes on the cool station while you do this step. ***The cells can now take up DNA!*** *

4. Transformation

90 minutes (immediately after prior step)

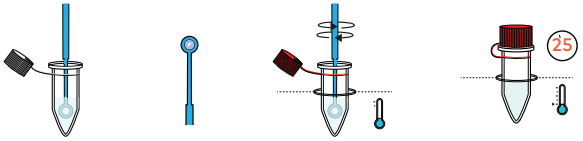
Goal

Introduce a DNA plasmid into competent bacteria to and allow them to recover in LB media



Materials from your kit

(1) DNA plasmid
(1-2) Competent cells [from prior step]
(1-2) Recovery media
(1-2) Blue loop (1 uL)

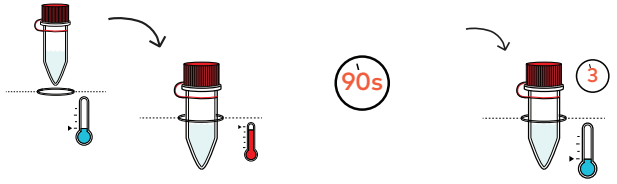


4.1 Dip a Blue inoculating loop into the DNA plasmid tube. When you pull the loop out of the DNA tube the hole of the loop should have liquid in it. Close the tube.

4.2 For each tube of competent cells on the 4˚C tube coolers, slowly dip and spin the inoculating loop containing DNA. Stir/swirl for **5 more seconds** to fully mix and place the tube back on the **4˚C station**. Discard the loop. * **Do not reuse the inoculating loop!** *

4.3 Incubate for **20 minutes** on the **4˚C tube coolers.** You can put the DNA tube back in the fridge.

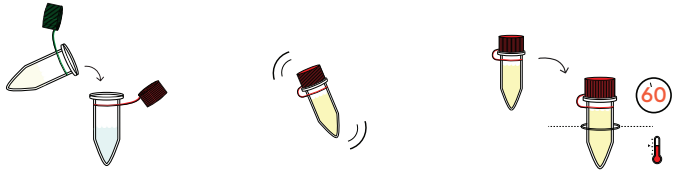
4.4 Turn on the **42˚C tube heaters,** wait for them to reach **42˚C**



4.5 Heat shock by inserting the buffer tube in the **42˚C tube heaters** for **90 seconds.** Time this carefully

4.6 Immediately place back on the **4˚C Tube Coolers** for **3 minutes**

4.7 Use the screen control to change the **Tube Heaters to 37˚C.**



4.8 Pour the tube of Recovery media (~300 uL) of liquid growth media to the cells and mix by gently inverting 10 times. Some liquid will stay in the recovery tube. That is acceptable.

4.9 Place the buffer tube(s) in the **37˚C tube heaters** for **60 minutes** to allow them to recover and start expressing their antibiotic resistance proteins. You can mix the cells by inverting them every 15 minutes. * **Make sure the cells are in the bottom of the tube when they are placed in the tube heaters!** *

5. Plating Cells

10-15 minutes + 24 - 72 hrs wait

Goal

Transfer your transformed bacteria onto selective LB agar plates in order to grow colonies of engineered bacteria

Materials from your kit

(1-2) Antibiotic Plates (Step 1)
(1-2) Recovered cells [prior step]
(1-2) Yellow loop (10 uL)

5.1 Turn on the **37˚C hot plate incubator**

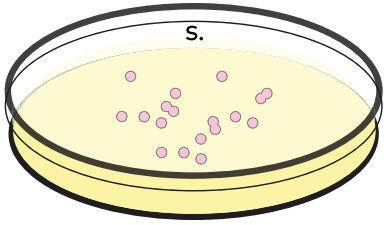
5.2 On the poured LB Agar plates with antibiotic (selective), pour 1/3 of your recovered cells and spread with a yellow inoculation loop. One plate for each transformation!

5.3 Let the plate stand for **10 minutes** with the lid on to allow the solution & cells to soak into the LB agar.

5.4 Once dried, place the plates in the **37˚C hot plate** incubator upside down for **24-72 hours**. Inverting the plates ensures that water vapour in the plates keeps the LB agar surface moist. **Careful, if there is still liquid LB pooling in the plate, wait a little longer until it evaporates.**

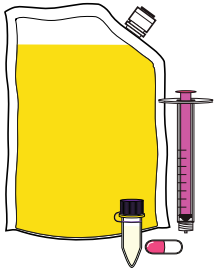
Did your cells grow ?

Goal
Verify if you have any cells that have grown overnight.



The cells will grow in colonies and start producing their new DNA program. If there aren't any colonies visible, wait another few hours before moving onto the Grow-it Kit..

Grow-it Kit Overview



The Grow-it Kit allows you to inject your transformed bacteria in the bioreactor area of the Amino One, a controlled environment for bacterial culturing.

A bacterial culture is a method of multiplying organisms by letting them reproduce in predetermined culture media, under controlled conditions. These can be used to study the organism or to create an abundance of them. The blank bacteria and the transformed bacteria of the engineer-it kit were grown on plates, which is one type of culturing.

Another method of culturing we will use here is liquid culturing, in which the desired bacteria are suspended in liquid broth (their food source). Cultures of lab-strain E.coli grow the fastest when there is a steady source of warmth (37˚C), agitation, and aeration.

Once your bioreactor is cleaned and filled with the food source and antibiotics, you will inject a single colony of your transformed bacteria through the inoculation port to inoculate the culture. The bacteria will reproduce at an exponential rate each 20 minutes and by hour 6 to 8 (depending on the initial colony size) you will see the clear yellow liquid turn cloudy. If your bacteria is programmed to change colour, the color will start appearing a few hours after. At this stage, you can start experimenting with the growth, monitor the sensor feedback, and continue feeding the culture for more and more growth! Each Starter Growth Kit comes with enough Food to grow bacteria for a 24-72 hour period—plenty of time for the DNA programs to begin expressing. If you wish to grow your bacteria for longer, food refills are available from our store. We recommend starting with 24 hour growth cycles.

Steps to culture your engineered Cells

1. Clean to sterilize the Amino One
   Day 3, 35-45 minutes

2. Load an Amino One with growth media & antibiotics
   Day 3, 10-15 minutes

3. Pick a colony and culture the transformed cells in the reactor
   Day 3, 5-10 minutes

4. Monitor growth and inject other liquid into the culture
   Day 4-5

5. Dispose and clean the Amino
   Day 6 or 7, 45-60 minutes

Each Grow-it kit has enough ingredients to grow 1 bacterial colony in a liquid culture. More Food bags can be used to keep the culture growing/producing longer.

Grow-it Kit components

Food : Lysogeny broth (LB) is the standard broth used to grow E. coli bacteria in liquid culture. This LB comes sterilized and ready to connect to your Amino One for a complete growth cycle.[1]

Antibiotics: For growth selection of your engineered bacteria, antibiotics are added to LB just prior to filling your Amino One. Amino Labs' antibiotic delivery system helps stabilize antibiotics for shipping and long-term storage. These capsules have a measured amount of antibiotics for 200 mL of LB. [1]

Inoculating syringe: This 3 mL syringe has the necessary connector to enable you to securely connect to the inoculating port on your Amino One.

Inoculating media: This is used to flush the bacteria out of the syringe and into your Amino One. This 6 mL of inoculating media is enough for a double flush.

Reusable cap/connector for Food: The reusable cap/connector for Food (LB) bags replaces the lid on the Bacteria Food (LB) bag in order to connect to the Feed A port on the Amino One. This is reusable, so keep it and simply sterilize it by placing it in distilled water in a dish and placing it in the microwave for 5 minutes.

[1] For education purposes only.

Clean-it Kit components

Cleaning Solution A: This is used as part of the two-stage inactivation procedure. Cleaning Solution A comes as a powder inside of a disposable bag and you simply fill it with distilled water. The final volume is between 400-500 mL and is enough for one cleaning cycle.

Cleaning Solution B: This is used in the second stage of the two-stage inactivation procedure. Cleaning Solution B includes a 20mL container and a syringe.

Inactivation Tablet: This is the third stage of inactivation. The Inactivation tablet is placed in the waste container just prior to culturing and is active for 5 days. This ensures that during regular use, any overflow is inactivated when it enters the waste container.

Reusable Cleaning Solution A Connector Cap: The reusable cap/connector for Cleaning Solution A connects to your Food and Custom port. This is reusable, so keep it—Solution A and a distilled water rinse will keep it sterile.

Reusable Rinse Bag: The reusable water bag has connector for Feed A and Feed B as well as a connector for the Rinse Syringe. This bag holds 1.5 L of distilled water and is used for all rinse cycles. Wash it with hot water and soap when you get it and rinse it well with distilled water.

Cleaning Solution A injection syringe: A 10 mL syringe used to draw Cleaning Solution A from the Cleaning Solution A bag and inject it into the inoculating port and airports of the Amino One.

Rinse Syringe: A 10 mL syringe used to draw distilled water from the reusable water bag and inject it into the inoculating port and airports of the Amino One during the rinse cycles.

Waste container: The Amino One Reusable Waste container is a closed 4 litre (1 gallon) container that connects to the Amino One Waste Tube. It is durable and can withstand the inactivation solutions to collect both waste and overflow when growing your bacteria in the bioreactor.

1. Short Clean of Amino One

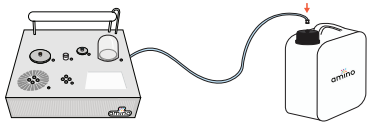
35-45 minutes

Goal

Make sure the Amino One system is sterile before your experiment by doing a short clean

Materials from your kit

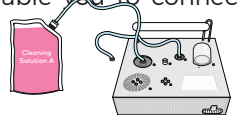
2L Distilled water (not included) (1) Sol. A reusable connector cap
(1) Rinse bag (Blue reusable bag) (1) Sol. A syringe & Rinse syringe
(1) Solution A bag



1.1 Make sure the a waste conatiner is connected to the waste tube **without any kinks in the tube and air is able to exit the container.**

1.2 Carefully fill the Solution A bag with distilled water. It will hold between 400-500 mL of liquid. You may want to use a funnel. Make sure to wear gloves as this is a strong chemical. Close the bag tightly to avoid leaks.

1.3 Attach the Solution A bag reusable connector cap. This cap has tubing that enable you to connect to several ports in your Amino One..



1.4 Let the bag stand for **a few minutes** with occasional mixing so that all the powder is dissolved.

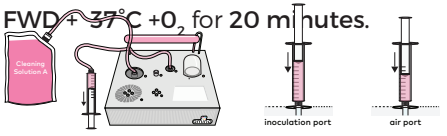
1.5 Connect the two white capped tubes on the Solution A cap to the **Food Port** and the **Custom Ingredients Port** intake tubes. One end remains free for later use.

1.6 Turn on **Food Port by pressing "Food"** and fill the chamber half way (if there is air left in the bag, it will be slowly sucked out by the Amino One prior to the liquid.

1.7 Turn on **Custom Port by pressing "Custom"** and fill the chamber the rest of the way. (Food turns automatically off if Custom is on)

1.8 Turn off the **Custom Port.**

1.9 Turn on **FWD + 37˚C +0_2** for **20 minutes.**



1.10 While circulating for 20 minutes, get the Solution A syringe, connect it to the available port on the Solution A bag connector cap and draw 10 mL of Solution A.

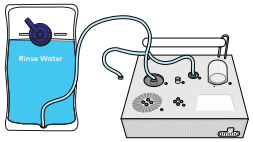
1.11 Slowly inject the 10ml into the **Inoculation Port**

1.12 Draw a further 10 mL of Solution A and slowly inject it into the **Air port**: the extra tube located under the large circle disk of the Food Port.

1.13 After the **20 minutes**, turn off **FWD + 37˚C +0_2** dispose of Solution A with the discard button.

1.14 Disconnect the Solution A bag and cap the tubes. The remaining solution can be kept to clean up surfaces, in your next clean cycle or poured in the toilet. The solution has a 5 day shelf life when mixed.

Rinsing

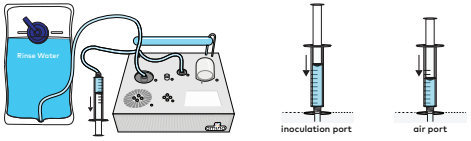


1.15 Fill the reusable rinsing bag (blue) with pre-boiled distilled water. It will hold 1.5 L

1.16 Connect the reusable rinse bag to **Food Port** and **Custom Port** intake tubes (as done for the Solution A bag)

1.17 Turn on **Food Port** for **30 seconds** and then **Custom Port** for **30 seconds.** Turn off Custom Port.

1.18 Turn on **FWD + 37˚C +0_2** for **5 minutes**



1.19 While the system circulates, get the Rinse Syringe and connect it to the rinse bag. Draw 10 mL water.

1.20 Inject 10 mL of water into the **inoculation port**

1.21 Draw an additional 10 mL of water, inject it into the **air port**.

1.22 After **5 minutes** of circulation, dispose of all liquid by pressing dispose on the screen.

1.23 Turn on **Food Port** and fill the chamber half way

1.24 Turn on **Custom Port** and fill the chamber the rest of the way. Turn off Custom Port.

1.25 Turn on **FWD + 37˚C & O2** for **5 minutes**

1.26 Repeat Rinse Syringes & Dispose. Steps **1.19 -1.22**

1.27 Repeat A Full Rinse Cycle: Steps 1.23-1.26

1.28 All liquids should be disposed and your reactor visibly clean and empty. The pH on screen should be above 4. If it is not, repeat a Full Rinse Cycle—some cleaning solution is left inside the system.

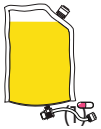
If you see any debris in the chamber, you can alternate with the FWD and REV flow direction to help dislodge.

2. Filling the Amino One

10-15 minutes

Goal
Add antibiotics to the LB media (to make it selective) and then fill the Amino One Bioreactor with this food.

Materials from your kit
(1) Bacteria Food (LB Media)
(1) Antibiotic Pill



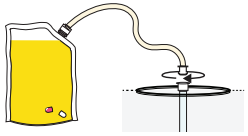
Reusable bacteria food connector lid

2.1 Get your sterile Bacteria Food pouch (LB media) and the antibiotic pill that matches the DNA plasmid that you transformed your bacteria with.

2.2 In a clean environment, open the sterile bacteria food pouch and pour the antibiotic pill inside.

2.3 Mix the LB around until the contents of the capsule dissolve (the gelatin capsule itself may not fully dissolve). You may have to squish the pill inside the bag to help release the contents. This can take up to 25 minutes.

2.4 After the contents of the pill are dissolved, replace the pouch lid with the Reusable Food Connector lid.



2.5 Connect the pouch to Food Port tube connector

2.6 Turn on Food Port until a thin layer of liquid is across the entire reactor chamber (this will take ~ 20 seconds)

2.7 Turn on FWD + 37˚C & O_2 for 1 minute. At this point you should see the pH increase to ~6.5.

2.8 Dispose of all liquids by pressing the dispose button

2.9 Fill the chamber $^2/_3$ full with the bacteria food (LB media). There will be some left in the bag and you can leave the bag attached to feed the culture later on.

2.10 Turn on FWD + 37˚C & O_2. This will heat up the LB to an ideal temperature for E. coli bacteria.

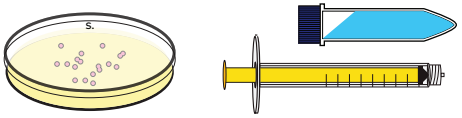
Your Amino One is now ready to start growing your engineered bacteria!

3. Picking a Colony & Culturing

5-10 minutes

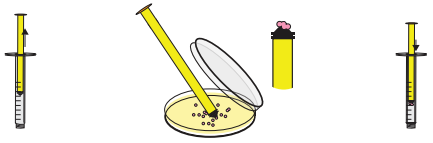
Goal

Inoculate the sterile LB media with a single colony of transformed bacteria.



Materials from your kit

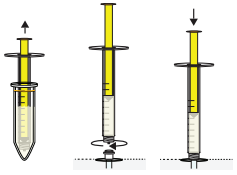
1) Plate with transformed bacteria (previous steps)
(1) Inoculation Syringe
(1) Inoculation Flush Solution



3.1 Pull the syringe plunger out of the syringe.

3.2 Open of the plate and touch the black tip of the syringe plunger to a SINGLE colony of transformed bacteria.

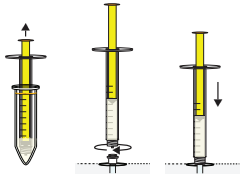
3.3 Place the syringe plunger back into the syringe housing.



3.4 Draw in 3 mL of Inoculation flush solution and connect the syringe to the inoculation port.

3.5 Rock the syringe back and forth 10 times (so that some bacteria on the syringe plunger go into the liquid.)

3.6 Slowly inject the liquid into the Amino One.



3.7 Disconnect the syringe and draw up an additional 3 mL of Inoculation Flush Solution.

3.8 Reconnect it to the inoculation port, rock it 10 times and then slowly inject into the system.

3.9 Place the cap back on the inoculation port tube.

Your bacteria will now grow and produce the DNA program over the next 24 hours. **FWD + 37˚C & O2 are turned on.**

4. Monitoring Growth & Adding New Ingredients

Goal
Monitor the growth of your organisms and modify their environment by injecting solutions

Materials from your kit
Solution of choice in a small container **(not included)**

4.1 To monitor the growth of your organism, keep an eye on the sensor data on the screen and on the WebApp. At first you won't see much with the naked eye, which is why the WebApp and on-screen sensors are a fun way to notice changes you can't see.

4.2 To inject something new into your culture you simply need to take the lid off of Custom Port connector and insert it into your solution.

4.3 Turn on Custom Port connector for the desired period of time. You'll be able to see pH and the population affected by the solution you're feeding in, if the new ingredient is of a different pH then that of the LB media / culture.

4.4 You can also feed more LB media through the Food Port to keep your bacteria growing as long as you want.

This is where you get to experiment! Share your results with the growing community @Aminobiolab.

If you decide to introduce new ingredients to the culture, it might affect your cell production prior to extraction.

If your experiment doesn't go as planned, or if you want to start fresh, you can use the same transformed (engineered) bacteria you made in the first part and get a new Inoculation & Growth Kit to repeat these last steps of bacterial culture to try out new things.

Congratulations!

By inoculating your culture with your transformed cells, and growing it over 16-24 hrs, you have succesfully bioengineered living organisms to produce DNA programs for you!

For the first 6-24 hours, you've keept an eye on your culture chamber and data—seeing cells growing, multiplying and maybe even changing colours.

If you had an App with an Extract-it Kit, go to the Extract-it Booklet section and continue your experience!

If you did not, and are ready to dispose of your culture to start a new one with a different bacteria (or store your Amino One), follow the directions for the Long Clean in the Clean-it Kit.

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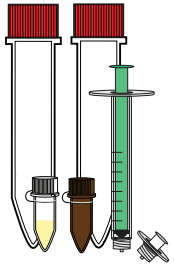
Extract-it Kit Overview



The Extract-it kit allows you to take the product created by your DNA progam plasmid (for example, a coloured protein) from within the bacteria so that you can use it for applications outside of Amino One. At the moment, this step requires access to a centrifuge, which is not included in the Amino One hardware.

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

Steps to extract proteins

1. Collect bacteria and centrifuge it down into a “pellet”.
   Day 6 or 7, 25-35 minutes

2. Lyse (break open) the bacteria using surfactant and enzymes.
   Day 6 or 7, 15 minutes with a 1-24 hour resting period

3. Collect and filter the pigments.
   Day 7, 10-15 minutes

Extract-it Kit components

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

Lysis Accelerator includes enzymes that break down the cell wall of bacteria and works with Lysis Buffer to release the contents of cells into their environment.

Collection Tube: This 15 mL tube is used to collect cells from the Amino One and to make them into a pellet using a centrifuge. If you don't have a 15 mL tube centrifuge, you can also add cells to the tube and let them sit in the fridge for 24-48 hours and the bacteria will settle at the bottom

Pigment Sterilization Syringe: This 3 mL syringe has the right connector to connect to the 0.22 um filter that is used to filter sterilize the pigments.

Sterilization filter: This filter has 0.22 um pores that lets pigments through and traps any remaining bacteria. Liquid that passes through the filter is sterile.

Pigment Storage Tube: This is a standard 1.5 mL screw top tube that can be used at the end to hold the extracted pigments.

Pigment Enhancer (optional): This tube contains nanoparticles that enhance the fluorescence of the pigments

1. Collect & “Pellet” the Bacteria

25-35 minutes

Goal

Collect bacteria in the collection tube and pellet them in the bottom

Materials from your kit

(1) Amino One culture
(2) Collection Tube
(1) Centrifuge for 15 mL tubes (~2,000 x g) (not included)

1.1 Instead of disposing of your culture in the waste container, open a 15 mL collection tube and fill it to between 10-14 mL full. Be careful not to spill on the counter! Use remaining Solution A to wipe any spills / contamination.

1.2 If there is an even number of groups doing this exercise, match up the amount of bacterial culture in the tubes (both should have the same amount). * It is extremely important to make sure the tubes have the same amount of liquid or the centrifuge will not be balanced and can break.*

1.3 Turn on the centrifuge to ~2,000 x g for 10 minutes. If you don’t have a 15 mL tube centrifuge, you can try filling your tube with bacterial culture and then let it sit on a counter or fridge. The bacteria may settle as a pellet in 24-48 hours

1.4 Pour off the supernatant (liquid on the top) into a waste container.

1.5 Add another 10-14 mL of culture to the tube.

1.6 Centrifuge for another 10 minutes.

1.7 Pour off the supernatant (liquid “floating” on the top) into a waste container.

1.8 You can repeat this 2-5 more times if you want to, but doing 2 cycles will be enough! If you have a 50 mL tube centrifuge, you can also use is to collect more cells faster.

Once your cells are collected for extraction, you can either :

A) Add more Food (LB) to grow a "new" culture from the cells remaining in the systems

B) Fully empty and clean the system for storage or to grow something new.

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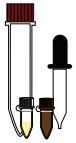
2. Lyse the Bacteria
15 minutes with 1-24 hour wait

Goal
Re-suspend the cells in lysis buffer and enzyme in order to break down the cell wall and release the product



Materials from your kit

(1) Tube with bacterial pellet (collection tube from previous step)
(1) Lysis Buffer tube
(1) Lysis Accelerator
(1) 1 mL Pipet

2.1 Once you have a bacterial pellet and all of the liquid is poured off into a waste container, add 1 mL of Lysis Buffer to the tube with a pipet and pipet up and down until the bacteria are fully suspended in the lysis buffer.

2.2 Pipet or pour the suspended bacteria into the brown tube with Lysozyme, put on the lid, and then invert 10 times.

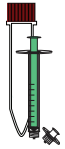
2.3 Leave this tube to incubate at room temperature for **1 - 24 hours.** During this period, the bacteria will be broken open and the pigments will be released into the solution. The longer the incubation time, the more product you will extract.

2.4 If you have a microcentrifuge, put the tubes into the centrifuge and spin at 13,000 x g for 10 minutes. If you only have the 15 mL centrifuge, spin at max for 10 minutes to pellet the cell debris. You may need to spin longer if you are using a 15 mL centrifuge.

*** Immediately move to the next step ***

3. Collect & Filter the Product
10-15 minutes

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



Materials from your kit
(1) Syringe
(1) Syringe filter
(1) New 1.5 mL tube

3.1 Attach the syringe filter to the syringe.

3.2 Pull out the syringe plunger from the cartridge.

3.3 Set the syringe and filter over a new 1.5 mL tube.

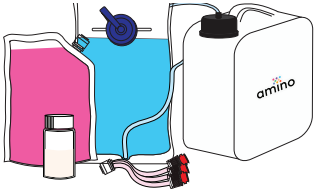
3.4 Pour the supernatant (extracted products) into the syringe (or use a pipette. Be careful to only pipet the clear solution!)

3.5 Put the syringe plunger back into the syringe cartridge and slowly press down. The products will pass through the filter. **Be careful** not too press to hard, because the filter could explode. If you did not effectively centrifuge the cell debris, the filter could get clogged.

3.6 You can now use your products! Follow up with cleaning your Amino One.

Share your creations with us ! @aminobiolab

Clean-it Kit Overview



Steps to sterilising the Amino One

1. Solution A Clean Cycle
   20 minutes

2. Quick Rinse Cycle
   10 minutes

3. Solution B Clean Cycle
   60 minutes

4. Long Rinse Cycle
   30 minutes

Clean-it Kit components

Cleaning Solution A is used as part of the Amino One two-stage inactivation procedure. Cleaning Solution A comes as a powder inside of a disposable bag and you simply fill it with distilled water. The final volume is between 400-500 mL and is enough for one cleaning cycle.

Cleaning Solution B is used in the second stage of the Amino One two-stage inactivation procedure. Cleaning Solution B includes 1 10 mL syringes and 20ml of Cleaning Solution B.

The Inactivation Tablet is Amino Labs third stage of inactivation. The Inactivation Tablet is placed in the waste container just prior to culturing and is active for 5 days. This ensures that during regular use, any overflow is inactivated when it enters the waste container.

Reusable Cleaning Solution A Connector Cap: The reusable cap/connector for Cleaning Solution A bag in order to connect to your Feed A and Feed B ports on the Amino One. This is reusable, so keep it—Solution A will keep it sterile.

Reusable Rinse Bag: The reusable water bag has connectors for Feed A and Feed B as well as a connector for the Rinse Syringe. This bag holds 1.5 L of distilled water and is used for all rinse cycles. Wash it with hot water and soap when you get it and rinse it well with distilled water.

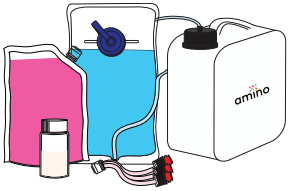
Cleaning Solution A injection syringe: A 10 mL syringe used to draw Cleaning solution A from the Cleaning Solution A bag and inject it into the inoculating port and airports of the Amino One.

Rinse Syringe: A 10 mL syringe used to draw distilled water from the reusable water bag and inject it into the inoculating port and airports of the Amino One during the rinse cycles.

Waste container (optional): The Amino One Reusable Waste container is a closed 4 litre (1 gallon) container that connects to the Amino One Waste Tube. It is durable and can withstand the inactivation solutions to collect both waste and overflow when growing your bacteria in the bioreactor.

1. Disposal & Clean of Amino One
120 minutes

Goal
Dispose and inactivate the culture and the Amino One.



Materials from your kit

2 L Distilled water (not included)
(1) Rinse bag (Blue reusable bag)
(1) Solution A bag
(1) Sol. A bag connector cap
(1) Solution A syringe, Solution B syringe & Rinse syringe
(1) Solution B bottle

1.1 After you are finished culturing your bacteria and want to dispose of and inactivate the rest of the culture, start by turning on dispose. This removes the majority of cells into the waste tank. Make sure the Amino One discard tank is connected to the disposal tube and that there are **no kinks in the tube.**

1.2 Carefully fill the Solution A bag with distilled water. It will hold between 400-500 mL of liquid. You may want to use a funnel and go slowly to avoid suds.

1.3 Attach the Solution A bag reusable connector cap. This cap has tubing that enables you to connect to several ports in your Amino One Personal Biolab.

1.4 Let the bag stand for **a few minutes** with occasional mixing so that all the powder is dissolved.

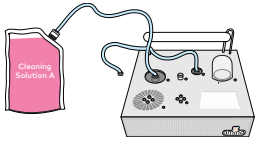
1.5 Connect the two white-capped ports on the Solution A connector cap to the **Food Port** and the **Custom Ingredients Port** intake tubes.

1.6 Turn on **Food Port** and fill the chamber half-way (if there is air left in the bag, it will be slowly sucked out by the Amino One before the liquid).

1.7 Turn on **Custom Port** and fill the chamber the rest of the way.

1.8 Turn off the **Custom Port.**

1.9 Turn on **37˚C + O_2 & FWD** for **20 minutes.**



1.10 While circulating for 20 minutes, get the Solution A syringe, and connect it to the available port on the Solution A bag connector cap and draw 10 mL of Solution A.

1.11 Slowly inject it into the **Inoculation port**.

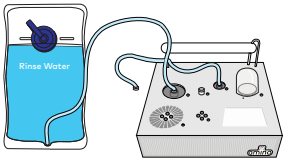
1.12 Draw an additional 10 mL of Solution A and slowly inject it into the **Air port** (the extra tube located under the large circle disk of the Food Port).

1.13 After the **20 minutes**, dispose of Solution A into the waste container.

1.14 Repeat steps **1.6 - 1.13**

1.15 Disconnect the Solution A bag and cap the tubes.

Rinsing

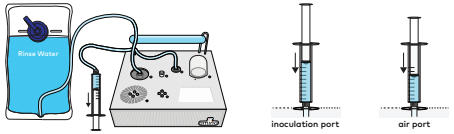


1.16 Fill the reusable rinsing bag (blue) with distilled water. It will hold about 1.5 L.

1.17 Connect the reusable rinse bag to **Food Port** and **Custom Port** intake tubes (as done for the Solution A bag).

1.18 Turn on **Food Port** for **30 seconds** and then **Custom Port** for **30 seconds.** Turn off **Custom Port.**

1.19 Turn on **37˚C + O_2 & FWD** for **2 minutes**



1.20 While the system circulates, get the Rinse Syringe and connect it to the rinse bag. Draw 10 mL of water.

1.21 Inject the 10 mL of water into the **inoculation port.**

1.22 Draw a further 10 mL of water, inject it into the **air port**.

1.23 After **3 minutes** circulation, dispose of all liquid.

1.24 Do a Full Rinse Cycle: Turn on **Food Port** and fill the chamber half way with water.

1.25 Turn on **Custom Port** and fill the chamber the rest of the way. Turn off **Custom Port**.

1.26 Turn on **37˚C + O_2 & FWD** for **5 minutes.**

1.27 While the system is circulating, repeat the Rinse Syringes & Dispose steps **5.20 -5.23**

Clean with Solution B

NOTE: Solution B is a chlorinated cleaner... be careful not to get it on your clothes, it will remove colours!

1.28 Turn on **Food Port** and fill the empty chamber half-way with water. Turn on **Custom Port** and fill to 2/3 full.

1.29 Turn on **37˚C + O_2 & FWD** for **60 minutes or longer.**

1.30 While the system is circulating, get a Solution B Syringe, draw 10 mL of Solution B from the bottle, and connect it to **the Inoculation port**. Slowly inject Solution B into the system.

1.31 Repeat this by refilling the Solution B Syringe and connecting it to the **air port** (under the large circle disk). Slowly inject into that port.

1.32 Turn on Reverse for 15 minutes

Second Rinsing

1.32 Repeat a Short Rinse cycle with steps **5.18-5.23**

Third Rinsing

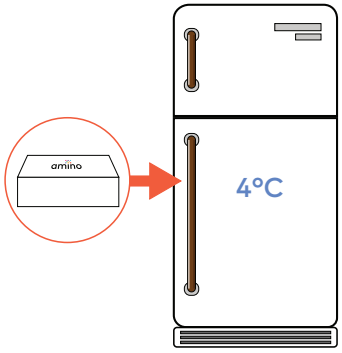
1.33 Repeat a Full Rinse Cycle: steps **5.24-5.27**

1.34 All liquids should be disposed and your reactor visibly clean and empty. See *After your Experiment* to dispose of the contents of the waste container.

After your experiments

Storing your work and remaining ingredients

After your experiment, you will notice that you now have blank cells, transformed cells, reusable consumables, and unused ingredients. You may now also have extracted products.



1. Replace the unused Ingredients, DNA Pigment tube, and Cell Stab tube in the box and place them in a refrigerator. You can keep them for 1 month.

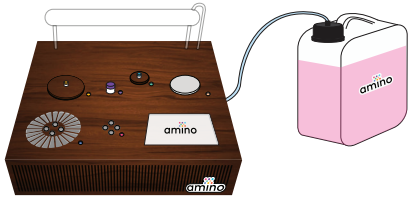
2. Dispose of your blank and transformed cells by placing them in a ziplock bag and half-filling it with bleach. Zip closed for 24 hours. After the wait, pour the liquid in the toilet and throw away the rest.

3. Take all the reusable connectors and place them in boiling water for 5 minutes to sterilize. Return them to ziplock bags and keep them with your Amino One for next time. The Reusable Water bag can be emptied and stored as well.

4. Your extracted products are stable at room temperature. Specific instructions, if necessary, will be given with different Apps.

Disposing of the Waste and Discard

To dispose of the inactivated content in the Waste Container, leave it inactive for 24 hours. This will allow the inactivation solutions to fully kill everything in the container. Then, simply pour it down the sink or toilet. Thanks to the inactivation tablet you added at the beginning and Solution A + B, all of the contents are completely inactivated and safe.



To dispose of the consumables used (loops, used tubes and syringes), use an old yogurt container or similar and fill it 1/3 with bleach. Open tubes and separate the plungers from syringes. Immerse for 24 hours, and then drain the liquid in the toilet and throw away the rest.

To clean the reusable items place them in a microwave safe container, fill it with distilled water, and boil it in the microwave for 5 minutes. Return them to their plastic bags and store.

Glossary

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

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

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

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

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

DNA: The DNA is the set of instructions that tells the cell how to function, like a computer program does.

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

Heatshock: When the cells are moved from ice-cold to warm temperature in order to take in DNA plasmids more efficiently.

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

Inoculating Loops: Inoculating loops are used to transfer liquids, cells and DNA from one vial to the next instead of traditional lab pipets, making your job easier, and less costly.

Non-Selective: A non-selective plate means that any cells put on this agar will grow.

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

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

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

Syringe: These needleless syringes are used to transfer calibrated liquids and inoculate the system, instead of costly lab pipets.

Transformation: See competent cells.

Troubleshooting

If the screen freezes, turn the Amino off, then on. At the moment, the Amino One doesn't remember where you are in your procedures; make sure to turn on all the functionalities you need when restarting it (make a note before you turn it off!)

If one of the input ports doesn't pull in, it might have some leftover cleaning agent clogging the tube. First, remove the connector at the end of the tube and replace it with the inoculation port connector. Using the rinse syringe from the Clean-it Kit, inject boiling hot water in the tube and wait a few minutes. Try to pull in again by turning on the port.

If you need to open the machine: you will need to open up the machine by unscrewing the top 4 screws (pop off the coloured screw covers). Slide the inner tray out of the wooden shell, take the top with the electronic / screen and flip it vertically so it gives you enough space to open up the fluidics top carefully. Disconnect the clogged tube from the main line and take it out of the inner tray. Using the syringe, push some boiling water through. Be careful, as it may cause pressure and spray. If you see some clogging you can massage the tube slightly while you push boiling water through. Once the tube is empty, reconnect it and reassemble the machine. Verify there are no leaks once you have reconnected the tube, before you put the wooden shell back on.

Emergency Discard Procedure: If for some reason the machine stops working and you have bacterial culture in the reactor, use the following procedure:

1) remove power cable
2) remove the back cover
3) gently tip the machine on its side with the discard tube down
4) pull the discard tube backwards so it is not kinked
5) using a syringe, repeatedly pump air into the airport (the port under the large disk). This will cause the culture to exit
6) once the culture has been removed, syringe in ~20 mL of Solution A
7) place the Amino flat in normal operating format
8) syringe in ~50-100 mL of Solution A
9) contact the amino team.

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