

GENETIC ENGINEERING WITH THE

Extract-it Kit - Liquid Culture™

————— User Manual —————

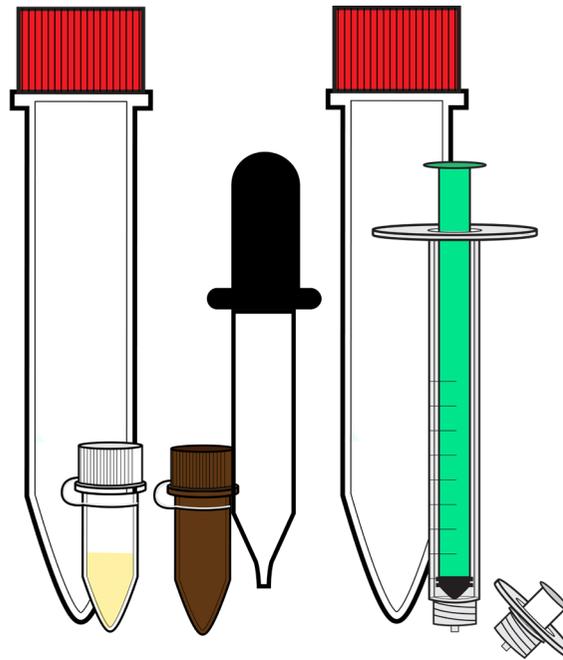


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Welcome! Let's get started

This User Guide was created to help you get the most out of your Amino Labs Experience.

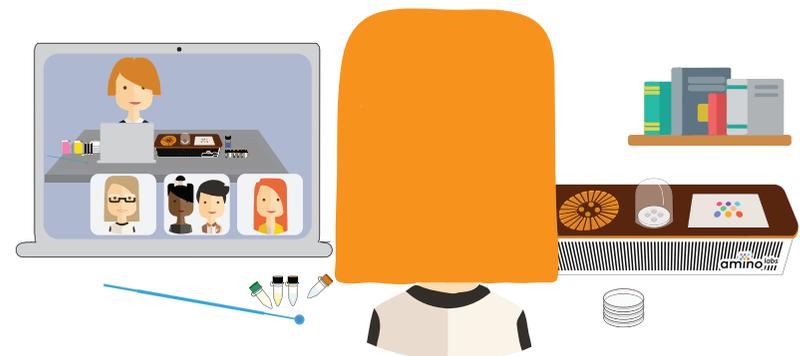
Even if you are familiar with bioengineering, science or other Amino Labs™ product, please take the necessary time to read through this guide. This will ensure you practice safe science, get the most out of your Extract-it Kit™ and finally, know what to do in case of a spill or accident!

This is a supplementary manual for the Extract-it kit™ alone, that supposes you have used and follow our DNA Playground with Engineer-it Kit™ manual, the Standalone Engineer-it Kit™ manual or the Amino One Bioproduction Lab™ manual.

This manual is procedural – these are the step by step instructions on how to run your experiment. Make sure to follow our tips! We will also cover "what's next", how to keep your creations, left over ingredients and clean up.

Amino Labs is very excited to welcome you to the world of the Biological Engineering Extraction with our 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!



Practice Safe Science

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

Firstly, the kit in your hands contains only non-pathogenic ingredients. These are rated Risk Group 1 (RG1) (Biosafety Level 1), the most benign level (and therefore the safest). With these ingredients, no special containment or training is required in North America*, but you must follow the following rules for your safety and the safety and success of your experiment(s)!

We recommend the system for ages 8+, under adult supervision, and 16+ unsupervised.

We recommend that the discard container be emptied by an adult and that the cleaning instructions be strictly followed for safety and experiment success. Make sure to store the ingredients in accordance with the instructions found in this booklet (refrigerated, in the freezer, or room temperature). Eyewear is not provided but should be worn.

- **Do not eat or drink near your experiments.** Keep your experiment at least 10 feet from food, drinks, etc. Under no circumstances should you consume any of the ingredients.
- **Immunocompromised persons:** While the ingredients in these kits are considered non-pathogenic, some persons, such as immunocompromised persons, can be affected by large numbers of bacteria and should wear extra protection to ensure no contact with the ingredients.
- **Wash your hands before and after** manipulating your experiment, the ingredients, or the hardware.
- **Wear gloves**, even when cleaning your station or handling the consumables (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. Also, after you put your gloves on, be aware of what you touch. Try not to touch your face, scratch itches with your gloved fingers!
- **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 spay cleaner will also work.
- **Find a container to discard used consumables** such as the blue and yellow loops, and tubes. An old large yogurt container, large plastic cup or the like will do. You will then fill this with bleach once your experiment is over, as described in the "After your experiment" section.

Equipment Needed

To use the Extract-it Kit you will need the following:

- **Microcentrifuge**
Spins at 13,000 RPM or greater
- **Large 15mL tube Centrifuge (Optional)**
Spins up to 4,000 RPM

Cleaning and safety supplies needed

- **Disposable container 500ml-1L** for waste (e.g. yogurt container)
- nitrile, (or similar) **gloves** like the ones found at a pharmacy.
- **Chlorinated bleach** (*mix a 10% solution: 1 part bleach to 10 parts water*) and a plastic bag to clean up.

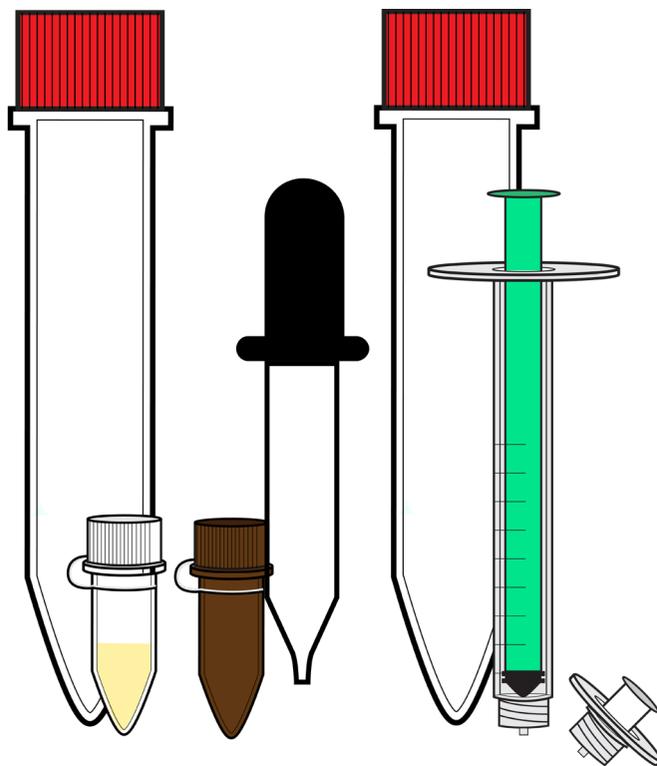


Setting up your space

Setting up your space is easy. Find a level, non-porous surface. Make sure the surface is bleach safe, and wipe it down with a solution of 10% chlorinated bleach or a chlorinated cleaner. Find and place a container of about 500ml-1L to collect your experiment waste : liquids, ingredient tubes, wipes. We suggest an old yogurt container or similar that you can fill with bleach to inactivate and then throw out at the end.

Make sure your centrifuge(s) is on a solid, level surface. Refer to the manufacturer's instructions for set up.

Discover your Extract-it Kit™



What does your kit allow you to do?

For an end-to-end bioengineering experience, Amino Labs provides you with the means to extract and purify what your bacteria produced so you can use it outside of the system. In other words, The Extract-it kit allows you to take the product created by your DNA program plasmid (for example, a coloured protein) from within the bacteria so that you can use it.

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!

Specifically, your kit will allow you to complete the following hands-on exercises to successfully achieve a protein extraction

1. Collect bacteria and centrifuge it down into a "pellet".
2. Lyse (break open) the bacteria using surfactant and enzymes.
3. Collect and filter the pigments.

What's in your kit?

Inside the Extract-it Kit™, you will find these components:



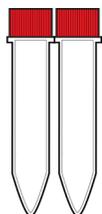
Lysis buffer: : softly breaks open (lyses) the cells to release the cell contents. This buffer should be used in concert with Lysis Accelerator.



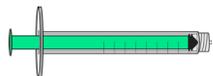
Lysis Accelerator: includes enzymes that break down the cell wall of bacteria and works with Lysis Buffer to release the contents of cells into their environment.



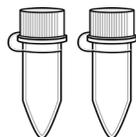
0.22 um filter : This filter has pore sizes that are 0.22 um which are smaller than bacteria. This means bacteria cannot pass through, but your pigment (smaller than 0.22 um) can.



Collection Tube : These 15 mL tube are used to collect and pellet cells from the Bioproduction Lab 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



Syringe: Used to push unfiltered extract through a filter. Caution! Do not press too hard to avoid liquid mishaps. Goggles recommended when using the syringe.



(2)1.5 mL Screw Cap Tube : Use one to store your final, extracted and filtered product, and one to use as a cell debris spin tube.



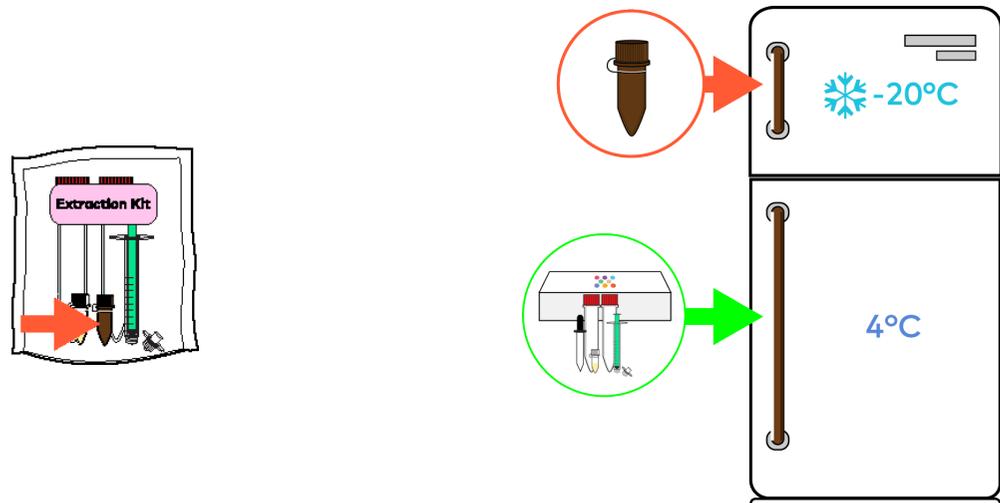
Pigment Enhancer Tube : Mix these nanoparticles with your filtered and extracted pigments to enhance their fluorescent properties, if applicable.



Pipet : Pipets are used to resuspend the cells in Lysis Buffer

Unpacking and Storing your kit

For a better shelf life and successful experiments, take the brown tube (Lysis Accelerator) out of your Extract-it Kit™, place it in the freezer and place the rest of the kit components in a standard refrigerator at around 4°C.



Technical Specifications

Lysis buffer: 1 mL

Lysis Accelerator: 5 mg

Filtration: (1) 0.22 um filter

Syringe: (1) 3 mL

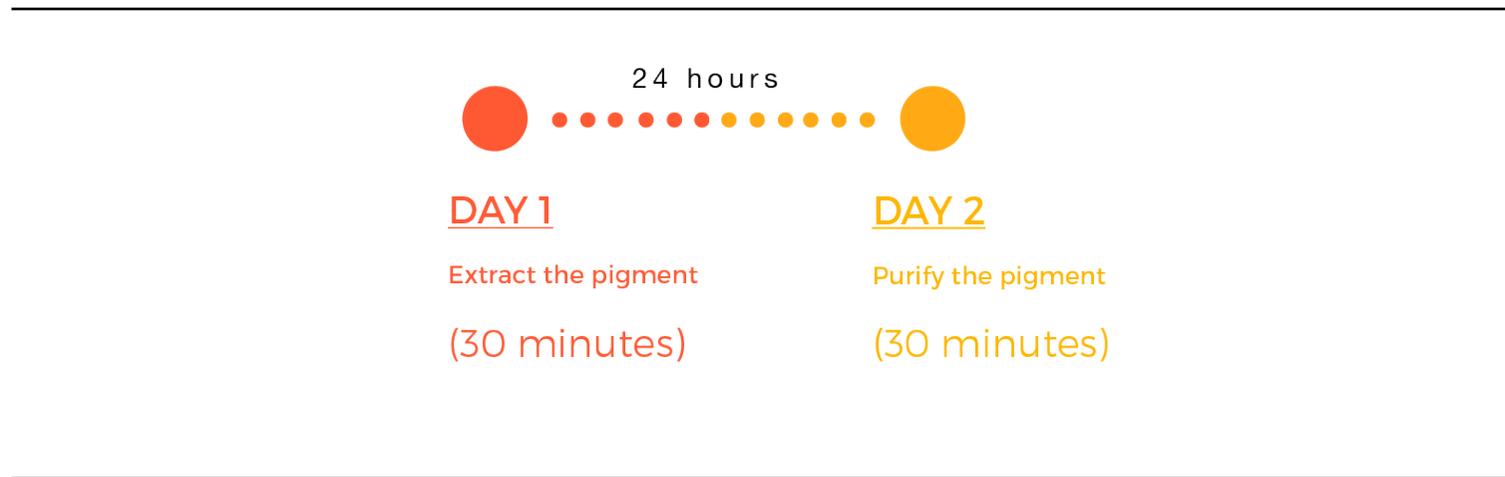
Tubes:

(2) 15 mL

(1) 1.5 mL Screw Cap

Implementation Timeline

The Engineer-it Kit™ takes 2 days of hands-on activity to complete, and 24 to 72 hours to see results.



3 "activities" or steps make up the Extract-it experiment:

1. Collect bacteria and centrifuge it down into a "pellet".
Day 1, 25-35 minutes
2. Lyse (break open) the bacteria using surfactant and enzymes
Day 1, 5 minutes with a 1-24 hour resting period
3. Collect and filter the pigment
Day 2, 10-25 minutes

Start your Experiment : *Experiment protocol*

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
(1) Collection Tubes

(1) Centrifuge for 15 mL tubes
(4000 RPM)

Note

If you do not have a Bioproduction Lab, you can grow your engineered cells from the Engineer-it Kit in a shaker incubator using LB media and the correct antibiotics in flasks. After growing in the shaker incubator for 24-48 hours, move to step 1.1.

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 4000 RPM 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 it to collect more cells faster.

If you have more than 50mL of culture, your cells may not extract efficiently.

Once your cells are collected for extraction from your Amino One Bioproduction Lab you can either :

A) Add more Food (LB) to grow a fresh culture from the remaining cells in the Bioproduction Lab

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

Experiment protocol cont.

2.

Lyse the Bacteria

15 minutes with 12-24 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 Accelerator |
| (1) Lysis Buffer tube | (1) 1 mL Pipet |
| | (1) 1.5 mL clear cell debris spin tube |

2.1 Once you have a bacterial pellet and all of the liquid is poured off into a waste container, pour the 1 mL tube of Lysis Buffer to the collection tube with pelleted cells. With the pipet, pipet up and down until the bacteria are fully suspended in the lysis buffer. * Try not to create too many bubbles! *

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

2.3 Leave this tube to incubate at room temperature for 12- 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. If you have the opportunity , invert / mix the tube during the incubation period. This will improve the lysis reaction.

2.4 After incubation and before centrifuging, pour your lysed solution into the clear spin tube. This will allow you to keep an eye on your solution as the cell debris pellets out of your pigment. Using your microcentrifuge, put the tubes into the centrifuge and spin at 13,000 RPM for 15 minutes.

*** Immediately move to the step 3 ***

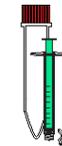
3.

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) 1.5 mL tube for final pigment |
| (1) Syringe Filter | |

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 the final pigment 1.5 mL tube.

3.4 Gently pour the supernatant (extracted products) into the syringe. This should be transparent solution, not cloudy. If cloudy, transfer it back to the tube and centrifuge for another 15 min. The cloudiness is cell debris and will clog the filter irreversibly.

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.

You can now use your products!

Share your creations with us ! [@aminobiolab](#)

After your Experiment



Using the Extract-it Kit™, you extracted and filter-purified a protein create by a DNA Program inside single-celled organisms, just like scientist and industries do every day inside their large laboratories. Great! We hope you enjoyed the experience, and will continue to experiment in the field of biological engineering.

For now, lets make sure you dispose of and store your remaining material correctly.

Storage, disposal and Clean up

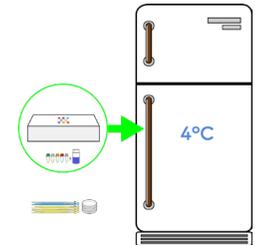
After your experiment, you will have cells, discard liquids and possibly unused ingredients.

1. Place the unused Ingredients in a sealed bag or box and place them in a refrigerator. Keep them for 1 month once opened. If unopened, the shelf life is 6 months

2. Dispose of your cells by placing them in a ziplock bag and half-fill it with concentrated bleach. Zip closed for 24 hours. Pour the liquid in the toilet and throw away the rest. You can also place them in your waste container if space is available. Use more bleach to wipe down your work area and equipment.

3. To dispose of the consumables used (loops, used tubes), fill your waste container 1/3 with bleach. Make sure the tubes are open. Immerse for 24 hours, and then drain the liquid in the toilet and throw away the rest.

4. Your extracted products are stable at room temperature. Keep them out of sunlight, like any other pigments.



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In no case shall Amino Labs, our directors, officers, employees, affiliates, agents, contractors, interns, suppliers, service providers or licensors be liable for any injury, loss, claim, or any direct, indirect, incidental, punitive, special, or consequential damages of any kind, including, without limitation lost profits, lost revenue, lost savings, loss of data, replacement costs, or any similar damages, whether based in contract, tort (including negligence), strict liability or otherwise, arising from your use of any of the service or any products procured using the service, or for any other claim related in any way to your use of the service or any product, including, but not limited to, any errors or omissions in any content, or any loss or damage of any kind incurred as a result of the use of the service or any content (or product) posted, transmitted, or otherwise made available via the service, even if advised of their possibility. Because some states or jurisdictions do not allow the exclusion or the limitation of liability for consequential or incidental damages, in such states or jurisdictions, our liability shall be limited to the maximum extent permitted by law.

SECTION 17 - INDEMNIFICATION

You agree to indemnify, defend and hold harmless Amino Labs and our parent, subsidiaries, affiliates, partners, officers, directors, agents, contractors, licensors, service providers, subcontractors, suppliers, interns and employees, harmless from any claim or demand, including reasonable attorneys' fees, made by any third-party due to or arising out of your breach of these Terms of Service or the documents they incorporate by reference, or your violation of any law or the rights of a third-party.

SECTION 18 - SEVERABILITY

In the event that any provision of these Terms of Service is determined to be unlawful, void or unenforceable, such provision shall nonetheless be enforceable to the fullest extent permitted by applicable law, and the unenforceable portion shall be deemed to be severed from these Terms of Service, such determination shall not affect the validity and enforceability of any other remaining provisions.

SECTION 19 - TERMINATION

The obligations and liabilities of the parties incurred prior to the termination date shall survive the termination of this agreement for all purposes. These Terms of Service are effective unless and until terminated by either you or us. You may terminate these Terms of Service at any time by notifying us that you no longer wish to use our Services, or when you cease using our site.

If in our sole judgment you fail, or we suspect that you have failed, to comply with any term or provision of these Terms of Service, we also may terminate this agreement at any time without notice and you will remain liable for all amounts due up to and including the date of termination; and/or accordingly may deny you access to our Services (or any part thereof).

SECTION 20 - ENTIRE AGREEMENT

The failure of us to exercise or enforce any right or provision of these Terms of Service shall not constitute a waiver of such right or provision. These Terms of Service and any policies or operating rules posted by us on this site or in respect to The Service constitutes the entire agreement and understanding between you and us and govern your use of the Service, superseding any prior or contemporaneous agreements, communications and proposals, whether oral or written, between you and us (including, but not limited to, any prior versions of the Terms of Service).

Any ambiguities in the interpretation of these Terms of Service shall not be construed against the drafting party.

SECTION 21 - GOVERNING LAW

These Terms of Service and any separate agreements whereby we provide you Services shall be governed by and construed in accordance with the laws of Ontario, Canada.

SECTION 22 - CHANGES TO TERMS OF SERVICE

You can review the most current version of the Terms of Service at any time at this page. We reserve the right, at our sole discretion, to update, change or replace any part of these Terms of Service by posting updates and changes to our website. It is your responsibility to check our website periodically for changes. Your continued use of or access to our website or the Service following the posting of any changes to these Terms of Service constitutes acceptance of those changes.

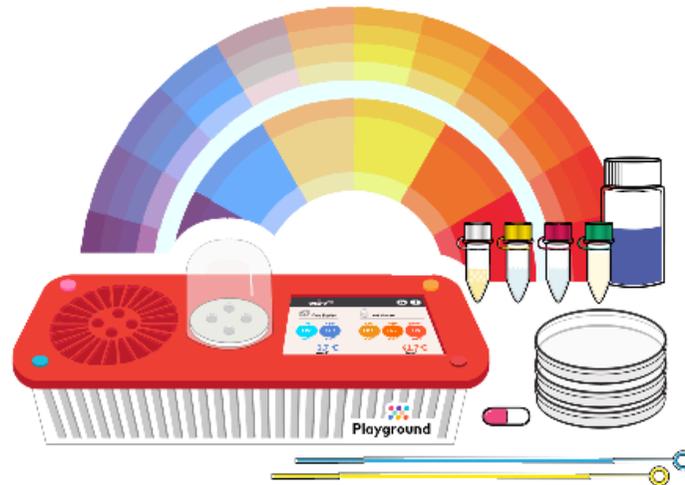
SECTION 23 - CONTACT INFORMATION

Questions about the Terms of Service should be sent to us at info@amino.bio

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