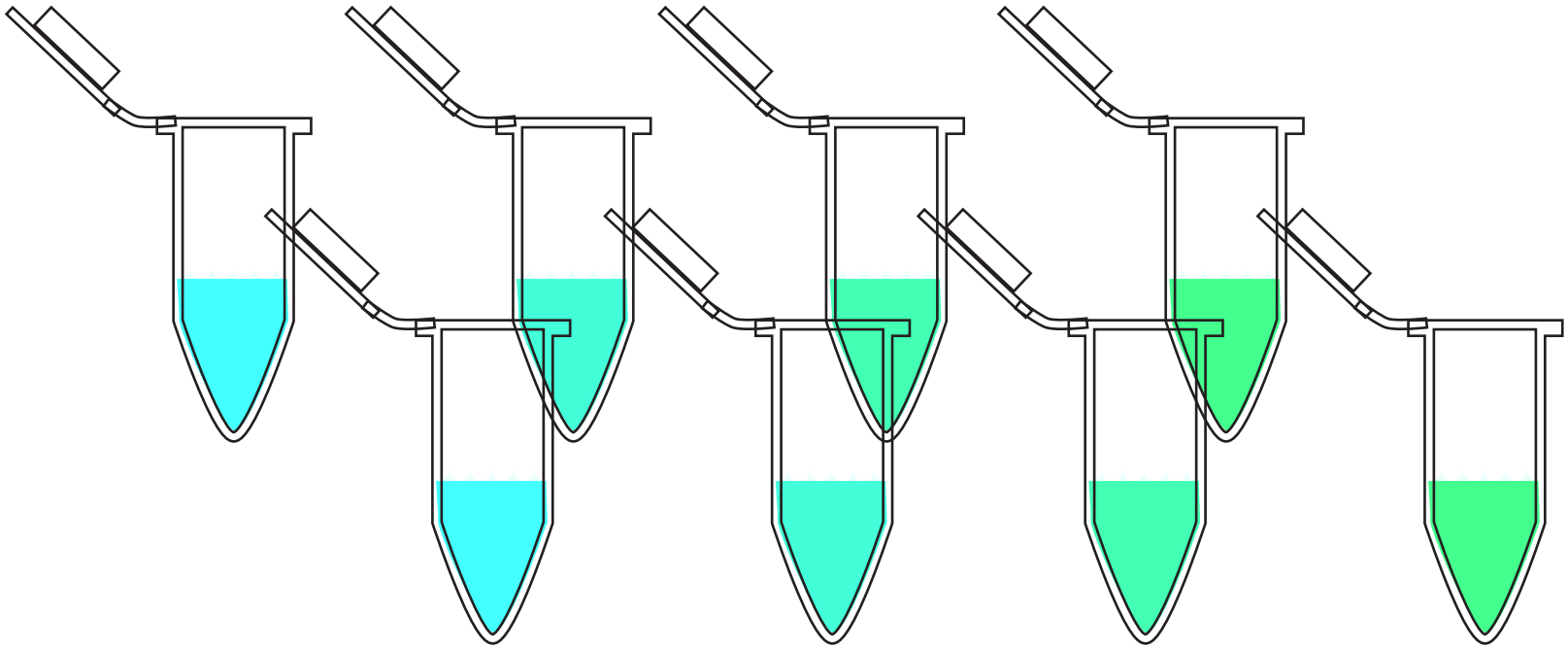
PCR-IT KIT™

CLASSROOM MANUAL



Version 1.2, Jan 2024

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Getting Ready - for educators

The Experiment - for educators & students

More information - for educators & students

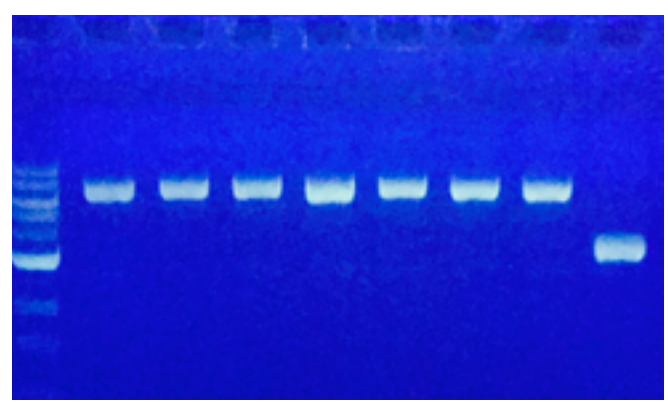
Welcome! Let's get started

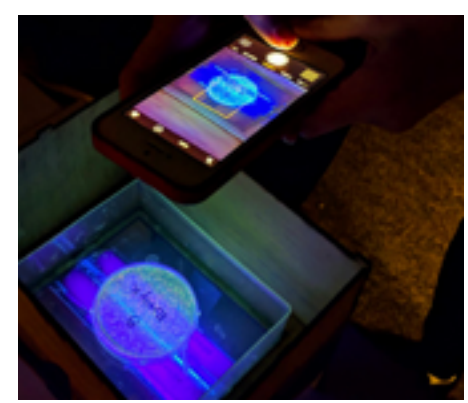
This user guide was created to help you get the most out of your Amino Labs experience. Even if you are familiar with genetic engineering, science or other Amino Labs™ products, please take the necessary time to read through this guide. This will ensure you practice safe science as well as store, use,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

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

Amino Labs is excited to welcome you to the world of the genetic engineering and DNA technology with our Zero to Genetic Engineering Hero™ journey, the PCR-it Kit™ and all our easy-to-use, easy-to-succeed at products!

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





Practicing safe science

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

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

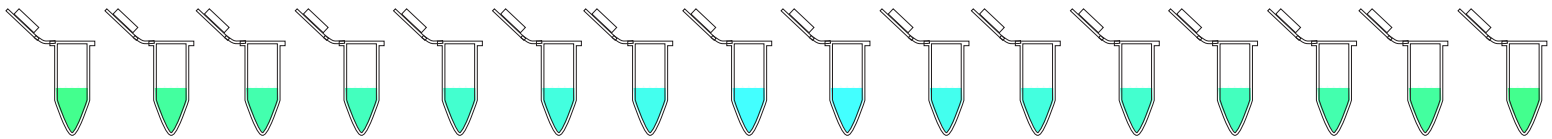
We recommend the system and kits for ages 12+, under adult supervision, and 14+ with or without supervision. We recommend that an adult empties the discard container. The cleaning instructions must be strictly followed for safety and experiment success. Make sure to store the kit per the instructions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

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

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

If you would like to do a short Online lab safety course for your edification, we recommend a Government of Canada

Discover the PCR-it Kit™



The PCR-it Kit™ has everything you need for 8 groups of students to undertake two PCR reactions each. These PCR reactions will each create two different DNA fragment sizes. Using the Agarose Gel Electrophoresis Kit or your own agarose gel electrophoresis supplies, your students will then run their samples in an agarose gel and analyze their results. You students have now learned to PCR!

The PCR-it kit is complementary with some of our other learning modules to bring a full, real-world science experiment to your classroom:

Amino Labs' PCR it Kit: Bioinformatics - Use software to calculate the theoretical sizes and sequences of the DNA fragments that are amplified in the PCR-it Kit (Free)

Amino Labs' Designing Primers: Advanced Bioinformatics - Use software to create primers to amplify a specific region of DNA from a DNA template (Free)

Amino Labs' Designing Primers for Cloning: Advanced Bioinformatics - Use software to design custom primers to amplify DNA for ligation with other DNA (Free)

Biomanufacturing & SOPs: Standard Operating Procedures, Quality Assurance, and Quality Control - Students undertake a role playing activity where they manufacture DNA fragments to specifications and read/write SOPs to maintain quality control of their manufactured DNA.

Kit components

Shared materials:

- **1 tube of pBR322 template DNA** (20 uL): pBR322 is one of the most commonly used *E.coli* cloning vectors
- **1 tube of pUC19 template DNA** (20 uL): pUC19 vector is a small, high copy number, *E. coli* plasmid.

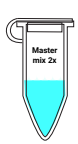
- **1 tube of Premixed pBR322 FWD/REV primers** (25 uL): Primers are short pieces of single-stranded DNA that are complementary to the target sequence, in this case pBR322. This premix contains both the forward and the reverse primer needed for your pBR322 PCR reaction.

- **1 tube of Premixed pUC19 FWD/REV primers** (25 uL): Primers are short pieces of single-stranded DNA that are complementary to the target sequence, in this case pUC19. This premix contains both the forward and the reverse primer needed for your pUC19 PCR reaction.

- **1 tube of Ultra water** (300 uL): Ultrapure water (UPW) is water that has been purified to high levels of specification. As a standard, the water contains only H20, as well as balanced number of H+ and OH- ions. To be classified as ultrapure, water must not contain any detectable endotoxins.

- **1 tubes of 6x gel loading dye** (40 uL): A gel loading dye is a colored dye that you mix with a DNA sample before running your sample in gel electrophoresis. Loading dyes are useful since they will make it easier to analyze your gel results since they add color to the sample, and they add density to your DNA sample, helping to prevent the DNA from being diffused into the buffer.

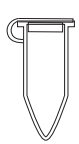
For each student group :



2/lab group

- **A bag of "2x Mastermix" PCR tubes:** A premixed, ready-to-use solution suitable for many PCR amplification experiment. It contains everything needed for a standard PCR reaction: Taq DNA Polymerase, dNTPs, Mg2+ and Reaction Buffer. Only primers and template DNA need to be added! **Each student group gets 2 tubes**.

2/lab group



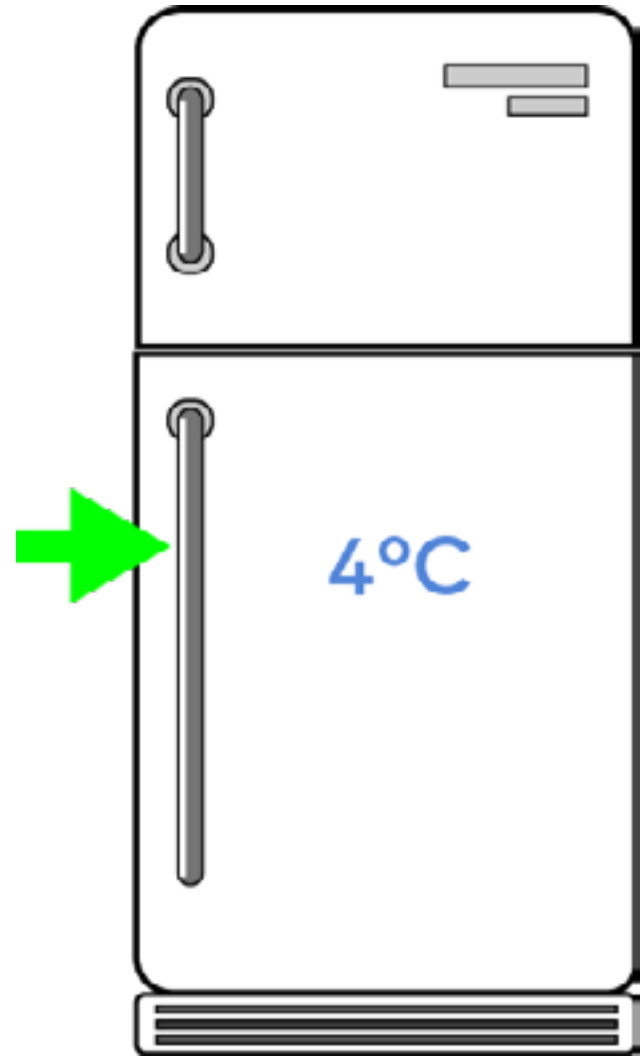
- **A bag of "Agarose gel loading" empty PCR tubes**: Small, sterile tubes with snap-shut caps made specifically for use in polymerase chain reaction (PCR) experiments. **Each student group gets 2 tubes**

Unpacking and storing kits

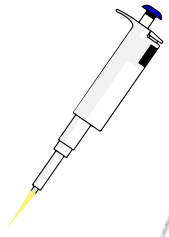
For a better shelf life and successful experiments, place your PCR-it Kit™ in a standard refrigerator at around 4°C.

If your refrigerator is not a science-only refrigerator, we recommend placing your science experiments inside a sealed plastic container before placing them in the refrigerator, especially once your kit is open.

Do Not Freeze your kit!



Necessary equipment



Equipment/Supplies required (not included in this kit):

- **Thermocycler/PCR machine:** 1 per class

- **DNA gel electrophoresis tank:** 1 per class or 1 per lab group
- **Power supply for electrophoresis:** 1 per class or 1 per lab group
- **Agarose gel casts and combs:** 1 set per lab group

- **1 Amino Labs Agarose Gel Electrophoresis Kit** or 1 agarose gel per lab group and matching buffer for your gel tank.

- **Micropipette (P10):** 1 box per lab group
- **Pipette tips (P10):** 1 box per lab group is ideal, but 1 box/class is enough tips to for the experiment.

Necessary safety supplies

Disposable container 500ml-1L
to hold tubes and other contaminated waste: 1 per station

Latex or nitrile gloves
like the ones found at a pharmacy. 1 pairs/student if students

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

Bleach ~500 mL
to inactivate all the experiment materials at the end of the experiment.

Timeline

This PCR Kit experiment is made up of 3 hands-on activities that can take place over 1 half day or 2 class periods. The experiment can also go further with an optional 3 periods for bioinformatics activities.

1. Getting your PCR Samples ready
Day 1, 20 minutes

2. Running your PCR samples in a PCR machine
Day 1, 90 minutes*

**If you are doing the bioinformatics activity, completing the first one while the PCR reaction is running works well.*

Bioinformatics activity 1 Calculating your DNA Fragment size and sequences: Use software to calculate the theoretical sizes and sequences of the DNA fragments that are amplified in the PCR reaction. **Complete this optional activity before running the gel.**

3. Analysing results with gel electrophoresis
Day 2, 60 minutes

Bioinformatics activity 2 Analyzing the DNA bands on your gel Generally measuring DNA requires expensive equipment that classrooms don't have. Use software to get a good estimation of the amount of DNA in a agarose gel **Advanced activity to be completed after running your gels.**

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Bioinformatics activity 3 Designing Primers: Use software to learn how to create primers to amplify a specific region from a DNA template. **Advanced activity that can be completed any time**

Recommended pre-labs

Amino Labs has many resources that should be used by your students before they complete the hands-on experiment to maximize their understanding and success. These pre-labs are meant to ensure your students know, understand, and complete all the experiment steps. Completing the pre-labs also minimizes the number of questions your students will have during the hands-on experiment.

1. Pipette-it Kit: Learn to pipette like a pro

Get the Kit or use your own supplies to follow the activity instructions

If your students are new to pipetting, this easy exercise can be done with or without the Amino Labs Pipette-it Kit. To complete the practice exercise, you will need pipettes, pipette tips, empty 0.5 and 1.5 mL tubes, water, and a scale.

2. Electrophoresis-it Kit: Learn gel electrophoresis

Explore the gel electrophoresis experiments kits

If your students are new to gel electrophoresis, we have several fun experiments that can guide them through learning to cast gels, running them, using ladders and analyzing their results. These experiment also includes background theory and quizzes to help make sure your students are gel electrophoresis pros!

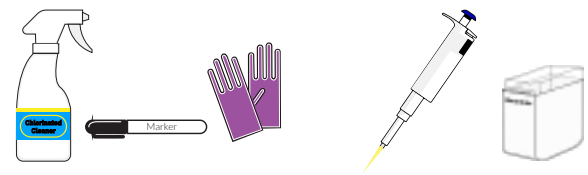
Teacher Experiment Setup

0. Prepare your classroom space

Goal Set yourself up for success.

Materials from your kit
Shared materials bag
Bag with empty PCR tubes
Bag with mastermix tubes

Materials not in your kit
Discard container (1 per table)
Permanent marker (1 per table)
Gloves (1 pair per student)
Chlorinated bleach spray or wipes
Paper towels
P10 pipettes and tips
PCR machine



Make sure the class has access to a microwave before starting as they will need this to make their gels if they are using the Amino Labs agarose gel electrophoresis supplies.

0.0 Have students download/print the manual and read through *Practicing safe science*, the *experiment protocol* and the *Glossary* pages.

0.1 Set your PCR machine up near the students work stations. Make sure the equipment is level and on a stable surface. Refer to the instruction manual to make sure you know how to use your equipment safely.

0.2 Set one permanent marker, one discard container and one P10 pipette with a tip box per work station on clean work surfaces. You can use chlorinated bleach spray, wipes or 10% bleach solution.

Note: If you do not have enough pipettes for all the student groups to have access to their own, set them up in a common area with the shared materials.

0.3 Distribute 2 tubes of Mastermix and 2 empty tubes to each student/student team. Keep the shared materials in a common area so all students can access it when needed.

0.4 Ask the student to use the discard container to dispose of:
- any used pipette tips,
- all empty tubes,

Paper, plastic packaging and gloves should be disposed in the regular garbage or recycling bin. After each day's experiment or at the end of the entire experiment, have students pour the content of their discard container into an inactivation bag (in the shared materials bag). Follow the instructions at the end of the manual to inactivate.

0.5 Ask the students to put on their gloves and wipe down their work surface with chlorinated bleach spray, wipes or 10% bleach solution if needed.

0.7 After the students complete the experiment, follow the *Storage, discard & clean up* procedures with them

If you are saving the tubes of DNA, primers, loading dyes, ultrawater and/or mastermix for a future experiment, place them back in their ziploc bag after use and refrigerate. We recommend you use a sealed plastic container to store all your experiment materials inside a refrigerator if you also use this refrigerator to store food or drinks. If you are not saving them, place the open tubes in a discard container and dispose of them after all the student teams have used them.

Student's Experiment Protocol

1. Prepare your PCR samples Day 1, 25 minutes

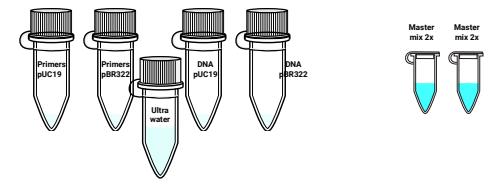
Goal Create the necessary mix of DNA and primers for the DNA you want to amplify.

Materials from your kit
(2) Mastermix tubes

Shared Materials
Pre-mixed F/R Primers pBR322,
Pre-mixed F/R Primers pUC19,
pBR322 DNA template,
pUC19 DNA template
Ultra water

Other materials
P10 pipette and tips
Permanent marker

Shared



Prepare

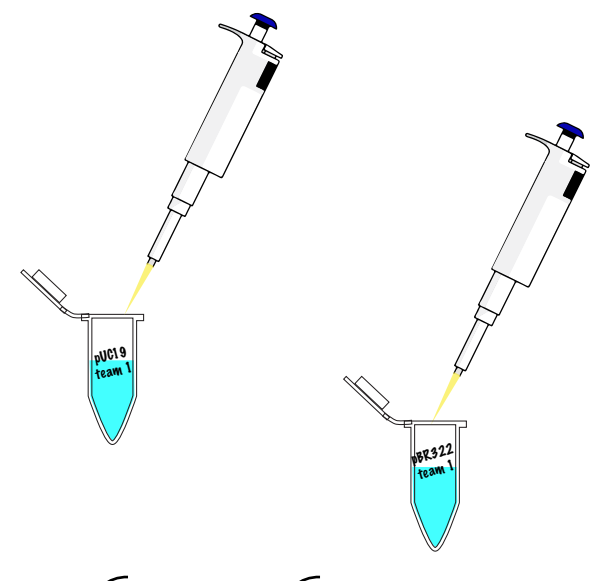
1.1 Locate your two empty pcr tubes and two 2x Mastermix pcr tubes or collect them from your teacher.

1.2 Use the permanent marker to label the two Mastermix tubes:
one with ***pBR322*** and one with ***pUC19***
also include your initials or team symbol so you can identify your tubes later on.

Add your reagents

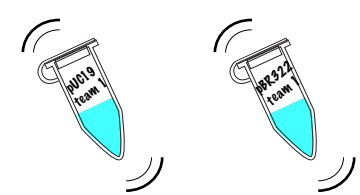
1.3 Using proper pipetting etiquette, add all the PCR ingredients to your tubes to ready them for a PCR reaction according to the table below.

Reagent	pBR322 tube (uL)	pUC19 tube (uL)
2x Mastermix (already in your tube)	12.5	12.5
Pre-mixed F/R Primers pBR322	2.5	0
pBR322 DNA template	1	0
Pre-mixed F/R Primers pUC19	0	2.5
pUC19 DNA template	0	1
Ultra water	9	9
Total volume of reaction	25	25



Mix your sample

1.4 After adding all the components together, mixed well by either pipetting up and down or by closing the tube and flicking it around, followed by centrifuging for 5-10 seconds.



2. Run your PCR samples in a PCR machine Day 1, 50 minutes

oal Create the necessary mix of DNA and primers for the DNA you want to amplify.

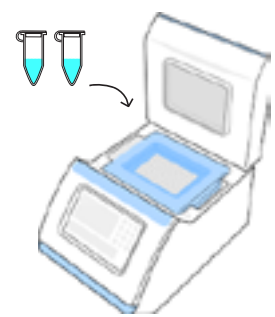
aterials from your kit
) Mastermix tubes with the
NA template, primers and ultra
ater added

Prepare

2.1 Make sure you have labelled your tubes so you can identify yours from other student groups

2.2 Place your tubes into a thermocycler (PCR machine) and program your PCR machine as follows:

Phase	Temperature	Time
Phase 1 (1 cycle)		
Initial Denaturation	**95 °C**	**2m:00s**
Phase 2 (30-35 cycle)		
Denaturation	**95 °C**	**0m:15s**
Annealing	**58 °C**	**0m:15s**
Extension	**72 °C**	**1m:20s**
Phase 3 (1 cycle)		
Final Extension	**72 °C**	**5m:00s**
Phase 4 (optional)		
Cool to 4 °C	**4 °C**	**∞**



Run your PCR samples

2.3 Run your reaction. The time to complete the reaction will be about 90 minutes depending but could be higher depending on your PCR machine.

Store your PCR reactions

2.4 Congratulations! You've just completed a PCR reaction and hopefully amplified some DNA fragements. The sample in your tubes are now called PCR products. You can store your PCR products in the freezer until you are ready to continue the experiment. If you have time (~1 hour), you can continue right away. Otherwise, you can keep your PCR products in the freezer for up to 1 year before completing the next step.

Bioinformatics Activity 1:
Calculating DNA fragment size and sequences 30-60 minutes

Goal Use software to calculate the theoretical sizes and sequences of the DNA fragments amplified in the PCR-it Kit

You will need:
Computer with internet access
ApE software (free)

Prepare

1.1 Install the ApE software on your computer by going to: https://jorgensen.biology.utah.edu/wayned/ape/

If you need, you can watch how to download and install the software using our quick youtube video.

1.2 Download the exercise file at amino.bio/pcr-it-bioinformatics-files

Watch the video & complete the exercise

1.3 Go to the this youtube link Amino Labs' Bioinformatics: Calculating DNA fragment size and sequences with ApE and watch the video to follow-along and complete the exercise. Click on the link to go to the youtube page.

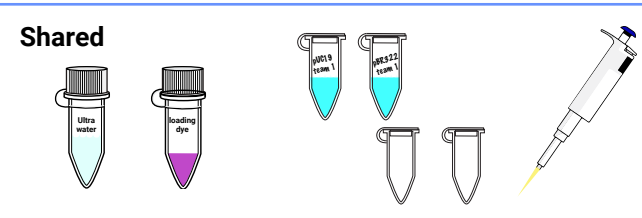
3. Analyze your PCR products in a gel Day 1 or 2, 60 minutes

Goal Analysing your amplified DNA Fragments using agarose gel electrophoresis to ensure your PCR was successful

Materials from your kit
Your PCR product from previous step
2 empty PCR tubes

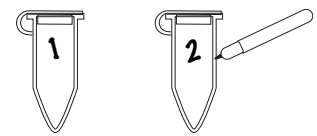
Shared Materials
Gel loading dye

Other Materials
P10 pipette and tips
from Amino Labs Agarose gel kit or your own supplies:
1 agarose gel for every 6 PCR reaction
1 kb ladder

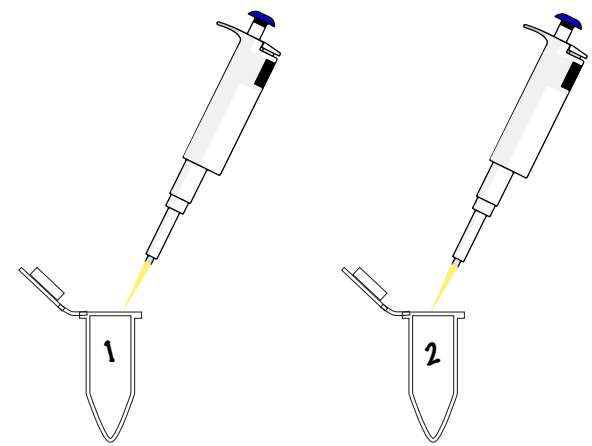


Prepare

3.1 Follow the instructions for Amino Labs Agarose gel kit or from your other agarose gel suppliers to cast agarose gels with a 8-tooth comb. You will be using two wells for your samples and one well per gel to include a 1kb ladder or similar. Up to three student can run their samples in the same gel.



3.2 Use the permanent marker to label the two empty tubes:
1 which you will use for pBR322 PCR product and ***2*** for pUC19 PCR product.



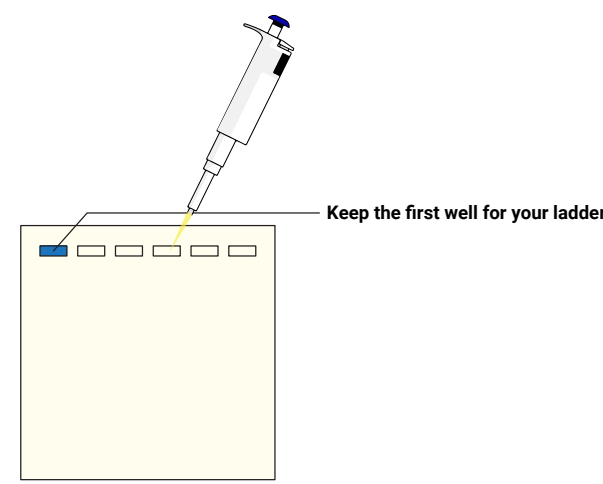
3.3 Use the following recipe to prepare your PCR products for the agarose gel:

Reagent	Tube 1 (in uL) for pBR322 PCR product	Tube 2 (in uL) for pUC19 PCR product
Ultra water	7.3	7.3
6x loading dye (usually blue or purple)	1.7	1.7
pBR322 PCR product	1	0
pUC19 PCR product	0	1
Total volume of reaction	10 uL	10 uL

Load your PCR products and ladder in a gel

3.4 Still following the instructions from your gel kit, add 10 uL from each of your prepared samples from your tube 1 and 2 to a well in an agarose gel. You can share the gel with other teams, but keep the first well of the gel free for your ladder. Remember to note in your lab book which sample is loaded where!

3.5 Add a 1Kb ladder (or similar) to the first well of the gel. Only one ladder is needed per gel!



Run your gel

3.6 Run your gel according to the instructions in your agarose gel kit.

Visualize your gel

3.7 Using your transiluminator visualize your gel to see if the PCR reaction worked as expected. We recommend taking a photo of your gel for further analysis. Make sure not to use any photo filters when taking the photo.

Bioinformatics Activity 2:
Analyzing DNA bands in a gel using software

Goal Use software to get a good estimation of the amount of DNA in agarose gel electrophoresis bands to estimate how much DNA you created in a PCR reaction.

You will need:
Computer with internet access
Fiji software (free)

Prepare

2.1 Install the Fiji software on your computer by going to https://fiji.sc/ or use the browser version of the software at https://ij.imjoy.io/

If you need, you can watch how to download and install the software using their tutorial video.

Watch the video & complete the exercise

2.2 Go to this youtube link: Amino Labs Bioinformatics: Analyzing DNA Bands with FIJI and watch the video to follow-along to complete the exercise.
If you are using the web browser version of ImageJ, note that @ 2:35 in the video, you'll need to click file in the upper left corner, and then open and select a local file. This will allow you to analyze a file on your computer and @10:20 you'll click analyze next to the process button, and then click measure.

Advanced Bioinformatics Activity 3:
Primer Design 30-60 minutes

Goal Use software to create primers to amplify a specific region of DNA from a DNA template

You will need:
Computer with internet access
ApE software (free)

Prepare

3.1 Make sure you still have the ApE software from the first bioinformatics exercise installed on your computer.

Watch the video & complete the exercise

3.2 Go to this youtube link: Amino Labs Bioinformatics: Primer Design Tutorial and watch the video to follow-along to complete the exercise.

Storage, Disposal, Clean Up

After everyone sees their results, all experiment materials including gels, tubes, and pipette tips should be in the discard containers. Disposing of experiment materials is an integral part of the experiment. **Always wear gloves for cleanup!**

A. **Reusable materials:** If you have DNA, primers, loading dye, ladders, ultra water it can last up to 6 months when stored in a refrigerator. Make sure it has not been contaminated before you store it. For example if you reused a pipette tip when pipetting from one of these tubes, it may now be contaminated. To store and keep these, place them in a ziploc bag inside a sealed plastic container in a refrigerator away from food items. If you do not wish to keep it, add to an inactivation bag. Make sure the lids are separate from the tubes so that the inactivating liquid can get inside. Follow the inactivation instructions below. If you are out of inactivation bags, use a sturdy ziploc type bag or disposable container with a lid. Always wear gloves when handling experiment materials and cleaners!

B. **Unused ingredients:** If you did not use all the mastermix tubes, store these for later use. Store them in their ziploc bag within a sealed container in the refrigerator for up to 6 months.

C. **Inactivation**: Transfer the tubes, gels, samples and pipette tips in the discard containers to an inactivation bag. Paper packaging, plastic bags, and gloves go in the regular garbage or recycling.

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

D. **Clean the discard containers** Spray some chlorinated bleach cleaner in the discard container(s) once emptied. Let it sit for an hour before wiping down. You can wait to wipe it down until you empty out your inactivation bags the next day.

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

Glossary

1 kB Ladder: A 1 kB ladder is a mixture of DNA molecules of different sizes that can be used as a reference when determining the size of DNA samples.

6x gel loading dye: a concentrated solution that is added to DNA or protein samples prior to loading them onto a gel for electrophoresis. The dye contains a tracking agent and a color indicator, which allows researchers to monitor the progress of their samples during the electrophoresis process. The 6x concentration is stronger than regular gel loading dye, so it is typically used for samples that are difficult to see or need to be loaded in a smaller volume. The dye is usually diluted with water or buffer before use.

25x TAE: 25x TAE (Tris-Acetate-EDTA) is a concentrated buffer solution used in gel electrophoresis to separate DNA fragments based on their size.

Agar: Agar is a gelatinous substance that is extracted from certain types of seaweed. It is commonly used in the laboratory as a growth medium for bacteria and other microorganisms. When mixed with nutrients and allowed to cool, agar forms a solid gel that provides a stable environment for microorganisms to grow. Agar is a popular choice for microbiologists because it is easy to work with and can be sterilized to prevent contamination. It is also relatively inert, meaning that it does not interfere with the growth of microorganisms or other cells.

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

Agarose: Agarose is a substance commonly used in molecular biology research made from a type of seaweed called red algae. The seaweed is harvested and processed to extract the agarose, which is a type of carbohydrate called a polysaccharide. Polysaccharides are long chains of sugar molecules that are important in many biological processes. Agarose is used in many different types of experiments because it forms a gel-like substance when it is mixed with water. This gel can be used as a medium for growing bacteria or other types of cells, but more often it is used to separate molecules based on their size in gel electrophoresis.

Agar vs agarose: Agar and agarose are related substances both derived from red algae. However, they have slightly different properties and uses in the laboratory. Agar is a mixture of various polysaccharides that is commonly used as a growth medium for bacteria and other microorganisms. It forms a solid gel when it is mixed with nutrients and allowed to cool, providing a stable environment for microorganisms to grow. Agarose, on the other hand, is a highly purified form of agar that is used primarily in molecular biology experiments. It forms a gel when it is mixed with water and is used to separate DNA molecules based on their size. Overall, agar and agarose are both useful in the laboratory, but they are used for different purposes and have different properties.

Anabolic Pathway: A series of reactions that builds more complex macromolecules from smaller molecules. Remember this by thinking "add" for anabolic.

Annealing: Annealing refers to the formation of a double strand DNA molecule from two complementary single strands of DNA.

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

Autoclave: An autoclave is a machine used to carry out industrial and scientific processes requiring elevated temperature and pressure in relation to ambient pressure/temperature. In life science, autoclaves are used to sterilize equipment and supplies by subjecting them to pressurized saturated steam at high temperatures (around 250 °F) for several minutes, up to an hour. Autoclaves are similar to baby bottle sterilizers and pressure cookers.

Bioinformatics: an interdisciplinary field that combines biology, computer science, and mathematics to analyze and interpret biological data. It involves the development and application of computational tools and algorithms to process, store, and analyze large amounts of biological data, such as DNA sequences, protein structures, and gene expression profiles. Bioinformatics plays a crucial role in many areas of biological research, including genomics, proteomics, and systems biology. It is used to predict the functions of genes and proteins, identify disease-causing mutations, design new drugs, and understand the evolution of species. Bioinformatics is an exciting and rapidly growing field that has revolutionized many aspects of modern biology and medicine.

Buffers: Buffers are solutions used to maintain a stable pH in a chemical or biological system. They help prevent large fluctuations in pH by absorbing excess acid or base. In the context of biology, buffers are used in many experiments, like DNA extraction, PCR, and gel electrophoresis, to ensure that the reaction environment remains within the optimal pH range for the reaction to occur. Different buffers may have different optimal pH ranges depending on the specific reaction or technique they are used for.

Casting gels: Mixing and boiling agarose powder and TAE buffer then pouring it into a tray to solidify. A comb is placed in the hot agarose liquid to form wells.

Catabolic Pathway: The opposing series of reactions to anabolic pathways. Catabolic pathways break down complex macromolecules from food into smaller molecules. Remember this by thinking "cut" as if the larger molecules are being cut into smaller ones.

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.

Cloning: cloning is a process used to create copies of DNA or cells. It is like making a photocopy of a document, but with biological materials instead. In molecular biology, cloning typically involves copying a DNA sequence, called a "insert" or "DNA fragment," and placing it into a vector, like a plasmid.

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".

Complementary sequences: These refer to Watson-Crick base pairing between nucleotides of separate DNA strands. In DNA, guanine (G) binds to cytosine (C),thymine (T) binds to adenine (A).

Denaturing: Denaturing is the opposite process of annealing and refers to the separation of a DNA double stranded molecule or the change in shape of a protein to a non-active form.

DNA: DNA can be thought of as a set of instructions that tells cells how to function just like a computer program tells your computer what to do. More specifically, DNA (Deoxyribonucleic acid) is a long, double-stranded molecule that carries genetic information in living organisms. The molecule is composed of four nitrogenous bases: adenine (A), guanine (G), cytosine (C), and thymine (T). The sequence of these bases determines the genetic code, which in turn determines the characteristics and traits of an organism. It is a fundamental molecule in the study of genetics and molecular biology, and plays a crucial role in various biological processes, such as protein synthesis and gene expression.

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

Gel Electrophoresis: In gel electrophoresis, a mixture of DNA fragments is loaded into wells in the agarose gel, and an electric current is applied. The DNA fragments move through the gel at different speeds, depending on their size, allowing them to be separated from one another.

Genome: an organism's complete set of genetic information, including all its genes. It is like a blueprint for the organism's development, growth, and function. Learn more about genomes in the *What is DNA?* simulator on amino.bio

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

Hybridizing: Hybridizing DNA strands is the process of two complementary single stranded DNA molecules annealing to each other forming a double strand.

In Vitro: The term for a biological process occurring in petri dishes and test tubes instead of inside an organism. For example, a PCR reaction utilizes the purified enzyme polymerase to build DNA strands however this procedure is done without using living cells.

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

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

Metabolic pathways: A series of linked chemical reactions known as anabolic and catabolic pathways.

Micropipette/pipette: A micropipette is a laboratory tool used to accurately measure and dispense small volumes of liquid. It consists of a pipette body, a plunger, and a disposable tip. With a pipette, you set the desired volume using a dial or button on the pipette, and then insert the tip into the

liquid. When the plunger is depressed, the liquid is drawn up into the tip by suction. The pipette is then moved to the destination, and the plunger is released to dispense the liquid. Micropipettes come in different sizes to measure volumes ranging from a few microliters (µL) to several milliliters (mL).

Monoculture: In a petri dish or liquid media, one type of cell is grown. The media is made specifically to the needs of the intended organism.

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

Organic compounds: Organic compounds are molecules that contain carbon atoms bonded to other atoms such as hydrogen, oxygen, nitrogen, and sulfur. They are typically found in living organisms and are often associated with biological processes. Organic compounds can have a wide range of functions and structures, from simple molecules such as methane (CH4) to complex molecules such as proteins and DNA. Some common types of organic compounds include carbohydrates, lipids, proteins, and nucleic acids. Organic chemistry is the branch of chemistry that studies these compounds and their reactions.

PCR: PPCR stands for polymerase chain reaction, which is a laboratory technique used to amplify specific regions of DNA. During PCR, short pieces of DNA called primers are used to target a specific region of DNA, which is then amplified through a series of temperature cycles. The cycles involve heating and cooling the sample, which allows DNA polymerase enzyme to copy the target region of DNA many times over. This results in the creation of millions of copies of the target DNA sequence, which can be used for a variety of applications, such as DNA sequencing, genetic testing, and cloning. PCR is a powerful tool in molecular biology research because it allows researchers to obtain large amounts of DNA from a small initial sample.

PCR machine: A PCR machine, also known as a thermal cycler, is a laboratory instrument that is used to perform the polymerase chain reaction (PCR) technique. PCR machines are designed to rapidly heat and cool samples of DNA, allowing researchers to amplify specific regions of DNA through a series of cycles. The machine is programmed to control the temperature of the sample in a precise and automated manner, which allows for consistent and reproducible results. The entire PCR process can be completed in a matter of hours, making it a rapid and powerful tool for a wide range of applications in molecular biology research.

PCR product: a copy of a specific piece of DNA that is made using a technique called polymerase chain reaction (PCR). During PCR, short pieces of DNA called primers are used to target and amplify a specific region of DNA, creating millions of copies of that region. The resulting PCR product can be used for a variety of applications, like DNA sequencing, genetic testing, and cloning.

Petri dish/ plates: A small plastic container used to culture (grow) bacteria in a controlled environment.

Polymerase: Polymerase is an enzyme that synthesizes DNA or RNA in the 3’ direction by adding nucleotides to the 3’ hydroxyl group.

Primers, Forwards and Reverse: To amplify any DNA sequence, two primers are necessary. One is called ‘forward primer’ and the other one is called ‘reverse primer’. The forward primer synthesizes the upper strand using the bottom strand as a template. Whereas Reverse primer uses the upper strand as a template and synthesizes the lower strand.

Recovery period: 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!

TAE (Tris-Acetate-EDTA): TAE is a buffer solution used in gel electrophoresis to separate DNA fragments based on their size. The buffer is made up of three components: tris, which maintains a stable pH; acetate, which provides ions for the current to pass through; and EDTA, which binds to metal ions and helps prevent DNA degradation. TAE is commonly used in agarose gel electrophoresis because it provides good resolution and can separate DNA fragments ranging from a few hundred base pairs up to several kilobases in size.

Template / Template DNA: Template strand is a strand of DNA to which a new complementary DNA strand can be synthesized from using the polymerase enzyme.

Transformation: Transformation is a genetic process by which cells take up foreign DNA from their environment and incorporate it into their own genome. In molecular biology, transformation is commonly used to introduce new genetic material into bacterial cells. This is achieved by making the cells "competent" or able to take up the foreign DNA, usually by treating them with a specific chemical or by exposing them to high voltage electrical fields. Once the cells are competent, the foreign DNA can be introduced into the cells by a variety of methods, such as electroporation or heat shock. The transformed cells can then be selected and cultured to propagate the newly introduced DNA. Transformation is a powerful tool for genetic engineering and is widely used in biotechnology, medicine, and agriculture.

Ultra water: Ultra-pure water is water that has been purified to an extremely high degree, typically through a process of filtration and distillation. This type of water is free from impurities such as dissolved minerals, organic compounds, and bacteria, and is commonly used in laboratory applications that require a high degree of purity.

Troubleshooting

Here's a short troubleshooting guide for some common problems that can occur during PCR:

No amplification: If you're not seeing any amplification of your target DNA, there may be a problem with your primers. Check to make sure they are designed correctly and annealing to the target sequence properly. You may also need to optimize the PCR conditions, such as the annealing temperature or the extension time.

Weak amplification: If you're seeing weak amplification of your target DNA, you may need to optimize the PCR conditions. Increasing the annealing temperature or the extension time may help improve amplification. You may also need to adjust the primer concentrations or the amount of template DNA in your reaction.

Non-specific amplification: If you're seeing amplification of non-target DNA, there may be a problem with your primers or PCR conditions. Check to make sure your primers are specific to your target sequence and not annealing to other regions of DNA. You may also need to optimize the annealing temperature or the extension time to reduce non-specific amplification.

Contamination: Contamination can be a common problem in PCR, especially when working with small amounts of DNA. Be sure to use sterile techniques when setting up your reaction and avoid cross-contamination between samples. Use separate pipettes, tips, and workspaces for each sample, and sterilize your equipment and workspace regularly.

PCR inhibition: PCR inhibition can occur when there are inhibitors present in your sample, such as proteins or salts. To avoid this, purify your DNA template before PCR and use clean, high-quality reagents for your reaction. You may also need to adjust the reaction conditions to optimize amplification.

Remember, troubleshooting PCR can be a complex process, and there are many factors that can affect the success of your reaction. By carefully optimizing your PCR conditions and troubleshooting any problems that arise, you can obtain high-quality results for your research.

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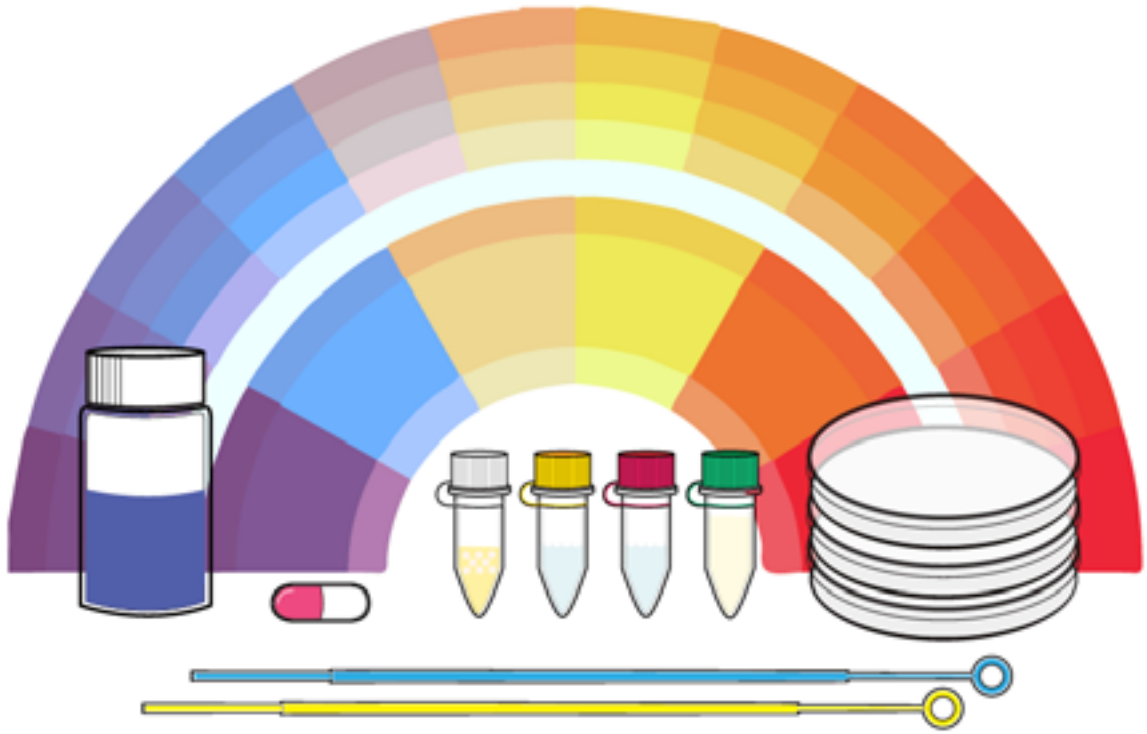

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