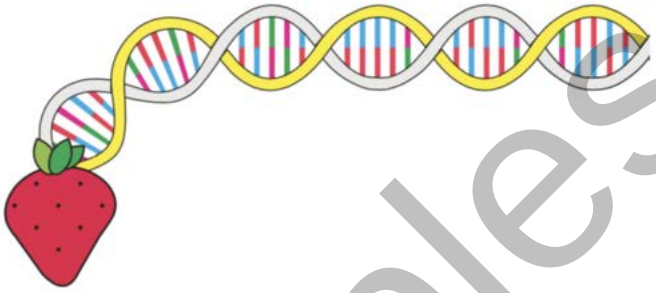
Chapter 1

Isolating DNA, the Blueprints of Life



Zero to Engineering Hero is unique in many ways from other biology books. So before we jump into the topic of Chapter 1, let's discuss how the chapters in this book are written.

This book is packed with hands-on exercises. Chapters start with the hands-on exercises so that you can "pull up your sleeves" immediately and start building genetic engineering skills. Within the hands-on exercise sections, there are "going deepers" and "break outs" that will provide more information about what is going on in the hands on exercise. You can read them, or skip them - you decide how much you want to learn. At some points you may feel like you need more background information, but trust us and continue on with the exercise to completion as there is a wealth of information later on in the Fundamentals.

Each chapter includes a Fundamentals section that covers many first principles at play in the hands-on exercise. This information is deliberately placed after the hands-on exercise so that you have real world skills and context to anchor the concepts in the Fundamentals. If during the hands-on exercise you are uneasy and want more, you can always start with the Fundamentals and circle back to complete the exercise, but we suggest doing the hands-on exercises first.

In Chapter 1, you will start your Genetic Engineering journey with a simple, engaging hands-on exercise: You will extract deoxyribonucleic acid (DNA) from a fruit. You have likely heard of DNA before in classic movies like Jurassic Park, forensic television shows or in the news. However, few individuals have ever seen DNA in real life or know how to extract it from an organims, living or dead. As you learn how to extract (separate) DNA from their cells and see it with the naked eye, you will also be learning the general principles of *Breaking Cells Open & Extracting DNA*. You may even be surprised at how much DNA you consume when you eat a single strawberry!

This exercise will help you to more richly understand the knowledge gained in the "Going Deeper" sections which focuses primarily on DNA, its structure and function. Further, by completing hands on exercise, you will begin laying the foundation for understanding and doing genetic engineering in the chapters to follow.

For this chapter, you do not need any specialized equipment and you can complete the exercise in your kitchen or at your desk. Chapter 2 will cover setting up the necessary equipment and space for the exercises in later chapters.

Getting Started
Equipment and Materials

You do not need any special equipment for this exercise. The materials can be obtained either by purchasing an ***DNA Extraction Kit*** online at ***www.amino.bio***, or by purchasing the components listed below at a local grocery store or pharmacy. The last two bolded items are not included in the ***DNA Extraction Kit.***

If you are eager to complete all of the exercises in this book, you can purchase a bulk collection of all the wetware kits: a ***Zero to Genetic Engineering Hero Kit Pack*** is available at ***www.amino.bio.***

Shopping List

99% isopropyl alcohol (rubbing alcohol) - 2 Tbsp
water (distilled, or bottled water) - 1 Tbsp
white salt - 1/4 Tsp
clear shampoo, hand or dish soap with EDTA (ethylenediaminetetraacetic acid) - 1/4 Tsp
1 paper coffee filter (#4 or #5 are a good size to fit in a cup)
1 very narrow glass, such as a shot glass
1 small sandwich ziploc bag
1 small drinking cup
1 soft fruit (1/2 kiwi, 1/5 banana, but 1 strawberry is best)

Instructional Overview

1. Make a saline solution in a ziploc bag
2. Mash up fruit in salt water to separate the fruit into individual cells
3. Add soap to lyse (cut open) the cells to release the DNA
4. Filter the lysed cell debris to isolate dissolved DNA
5. Precipitate (seperate) the DNA in alcohol so it becomes visible

Chapter Timeline Overview

What is DNA Breakout exercise : 15 min

The DNA Extraction Hands-on excercise: 20 minutes.

The Fundamentals section includes 2 breakout discussion / exercise.

What is DNA? *Breakout Session*

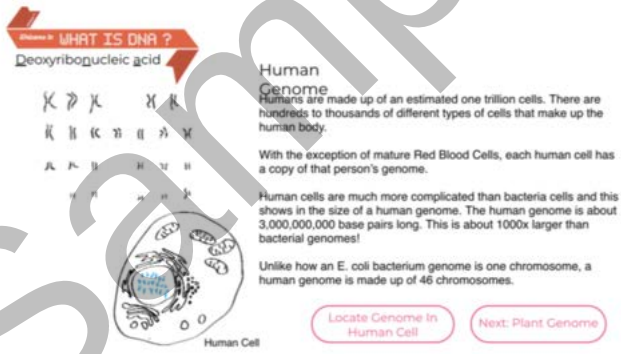
While you wait for your *DNA Extraction Kit* to arrive, or if you'd like to prepare with some contextual exercises on DNA prior to the exercise, you can get going now!

Grab your laptop or head to your desktop computer and go to the URL:
https://amino.bio/what-is-dna *note: not available on mobile devices.*

What is DNA? is a simple exercise that will provide you with basic context and historical facts about DNA in relation to the genome. You can do the exercise as many times as you like or need, and at any point during this book. We've found that a few tries at the drag-and-drop exercise really helps grasp what genomic DNA is.

In this chapter's hands-on learning activity, you will be getting a piece of fruit like a single strawberry and mashing it up so that the cells that make up the fruit become separated. You will then break open these cells to release the inside components of the cells into the outer environment. This includes releasing the cell's DNA, whereafter you will use some simple filtration and chemistry to cause the cells genomic DNA to become visible.

In other words, you will be extracting the genome from the strawberry cells, so knowing what a genome is will be helpful! You can find out now thanks to *What is DNA?* and/or you can find further information in the Fundamentals sections found after the hands-on learning.



Surfactants *Pro-tip*

There are many different kinds of surfactants used when doing genetic engineering experiments. In this exercise you used SLS—a common surfactant in hand soaps. In laboratories, a very common surfactant is called sodium dodecyl sulfate, or, SDS. Another popular surfactant which you will use in later chapters is called Triton X-100. In general you can browse the internet to find which surfactant will work for your experiment. In many cases you can use a number of surfactants for a particular experiment. For example, we could have used Triton X-100 for this exercise. We did not because Triton X-100 is much more expensive and harder to get then household soap, which provides the same result in this case.

Step 4. Filtering the cell debris

You now want to separate the DNA that is still dissolved in the salt water from the rest of the cell "debris" and micelles that you are not interested in for this exercise. This debris includes the carbohydrates and lipids from the walls of the cells, soapy micelles with cell debris, as well as clumps of proteins. Simple filtration will be sufficient to separate this debris and micelles from the dissolved DNA.

Set a **paper coffee filter** in a cup and pour the contents of your bag into the filter. Allow a few minutes for the salt water liquid containing DNA to pass through the filter. As the water, salt, and dissolved DNA pass through the filter (accompanied by some excess shampoo components and a small amount of proteins), the remaining molecules from the fruit will get caught in the filter. At the end of this step you'll have dissolved DNA in slightly soapy salt water in a cup. You need only ~1/2 Tsp of filtered "DNA" for the next step as there is plenty of DNA in that volume of liquid. Note that If you add too much filtered DNA liquid in the next step, the precipitation may not work as well - the next section will explain why.

You will notice that it looks just like red water, however. How do you know that DNA is actually in there? Lets make it visible!

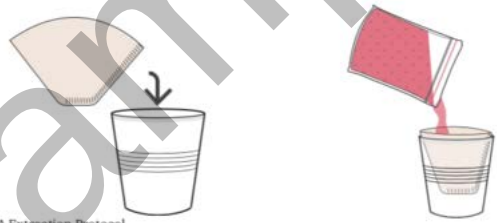


Figure 1-8. Step 4 of DNA Extraction Protocol

Step 5. Precipitating the DNA

In this last step, you are going to use chemistry to cause the DNA to "fall" out of the solution so that it can be seen with the naked eye. This "precipitation" is one of the most commonly used techniques in chemistry. Get your tube with **99% isopropyl alcohol or add 2 tablespoon 99% isopropyl alcohol** to a narrow/small glass and set it in front of you. You're about to see some chemical wizardry!

If you look at the liquid that passed through the filter, which scientists call the filtrate, you will notice it is clear and tinted red thanks to some red pigment

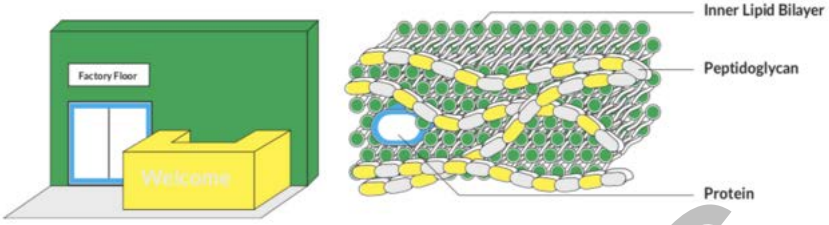


Figure 3-21. Factory lobby vs. the Intermembrane Space of a cell. At your back is the outer brick wall and outer membrane. You can see the Inner wall / Inner Membrane space just beyond the lobby.

The Lobby (C)

As we move into the factory, we pass through doors into the lobby (Figure 3-14). Cells also have doors, in a way. Some tubular-shaped proteins cross through the cell membranes and act as "portals", serving as the entry and exit points of the cell. This is where food enters the bacteria and waste exits the cell. We will be learning in-depth about proteins very soon.

Once you enter the lobby (Figure 3-21) you have also entered the building. However, you are not yet on the factory floor. The lobby is a transitional location and acts as an extra check point. In this lobby there is a security guard keeping an eye open for any trouble. The temperature, humidity, and air quality in the lobby is controlled, but may be different than the factory floor. Nevertheless, it is much closer to those conditions than the outside environment.

The lobby-like space of K12 E. coli is reached by passing through the outer membrane. This 'lobby' is called the inter membrane space (Figure 3-14). It is the space that exists between the outer cell membrane and the inner cell membrane. It is also a 'security check point' since most molecules that enter or exit the cell must go through this space. In the inter membrane space, there is an additional physical barrier called the peptidoglycan. Peptidoglycan, is a mesh-like protective barrier between the inner and outer membranes of E. coli cells (Figure 3-19 and Figure 3-21). The chemical environment of inter membrane space (salt concentrations, water molecules, and other cellular molecules) is more similar to the inside of the cell than to the outer environment. In other words, the intermembrane space is a transitional space that helps to maintain a controlled environment inside the cell.

The Inner Walls (D)

Have a look around this beautiful lobby - we can very clearly see the outer wall we just came through. We can also see a number of inner walls and passageways. These inner factory walls also play a very important role. Some parts of the factory may need to be warmer, colder or more humid than the lobby. These inner walls and passageways help to regulate the conditions in these different areas while contributing to the structural integrity of the building. (Figure 3-22).

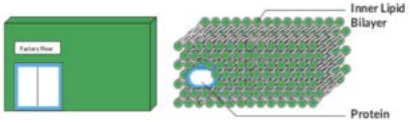


Figure 3-22. Factory Inner Wall vs. the Inner Membrane of a cell. You have passed though the lobby.

Peptidoglycan *Going Deeper*

Peptidoglycan is made of sugars (N-acetylglucosamine) that are connected to amino acids (alanine, glutamate, alanine) to form the mesh. As you now know, cells can create hybrid macromolecules. These are a combination of different macromolecules, for example a sugar bound to a lipid (to make LPS).

Peptidoglycan is a mix of sugars and amino acids. Over billions of years cells have been doing 'molecular mash-ups' to find new and interesting molecules that help them survive. What is really cool, is that they are almost exclusively made up of CHOPNS!

and fun" state. All of the anticipation and fear have transformed into the feelings exhilaration and fun. You also experience some relief, another by-product of the roller coaster reaction. The key parts of the reaction were the reactants (you as "anticipation and fear"), the energy needed to start the chemical reaction called the activation energy (the roller coaster car going up), and the products (exhilaration and fun, with some scream and relief). In the case of most chemical reactions that happen in cells, there is not enough activation energy, usually in the form of heat, in the cells to cause the chemical reaction to occur on its own. This is where protein enzymes become important.

Protein enzymes are 'magical' macromolecules that can 'catalyze' the chemical reaction. This means that even though there isn't enough energy, they can still cause the reaction to happen - they have a nifty "hack" to make the reaction happen. In technical terms, the "hack" is that the enzyme catalyst lowers the 'activation energy' required to cause a chemical reaction. This can catalyze a chemical reaction that wouldn't normally happen.

Each enzyme has a specific purpose and can catalyze a specific chemical reaction. This is what makes biology so special. There are thousands of protein enzymes that catalyze the chemical reactions necessary for life, including the creation of enzymes to catalyze more chemical reactions. Chapter 6 covers this in-depth

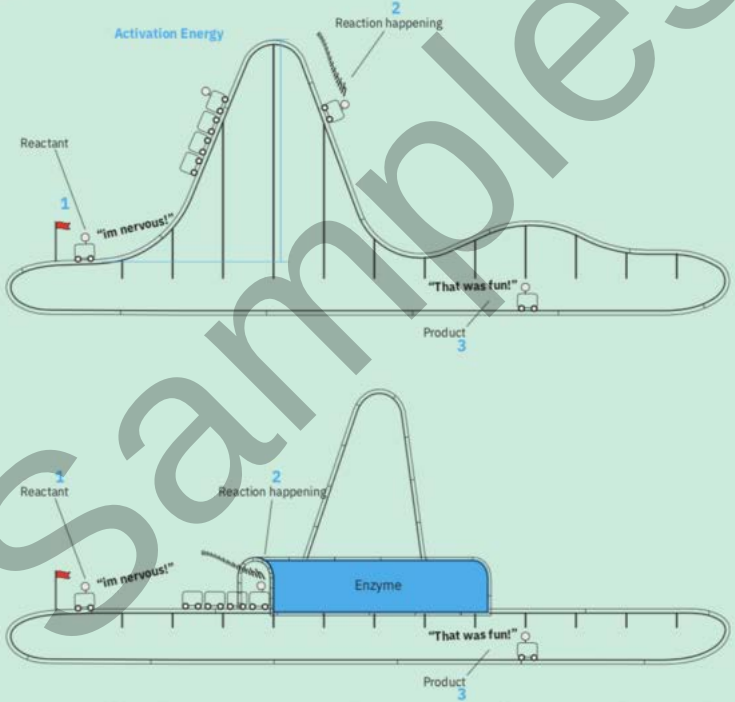


Figure 3-27. A normal chemical reaction requires reactants and activation energy in order to turn into products. Enzymes lower the amount of activation energy needed an cause reactions to happen in cells that wouldn't normally happen.

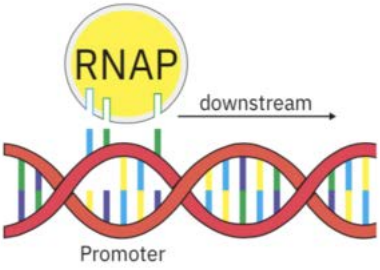


Figure 4-26. RNA polymerase can bind to the DNA nucleotides in one way which points the RNA polymerase in the correct direction.

During transcription: Which DNA strand does RNA polymerase read?

There are lots of nucleic acid strands to keep track of! During transcription there are three nucleotide strands involved:

- The RNA strand that is being made (transcribed) by RNA polymerase
- The two complementary DNA strands that make up the DNA double helix, one of which is being read by RNA polymerase

How does RNA polymerase know which strand of DNA to bind to so that it goes in the right direction?

The RNA polymerase binds to both strands simultaneously, but the direction it is pointing depends on the strand that the promoter sequence is in. In Figure 4-27 you'll see that the promoter (blue region) can actually be situated on either strand, with the arrows pointing in the downstream direction indicating the direction that the RNA polymerase would travel.

Whichever strand the promoter sequence is situated in is called the 'plus' (+) strand or 'Leading Strand' and the RNA polymerase will be oriented so that it will transcribe downstream of the promoter into the coding region.

Here's where things get a little wacky - ready for a mind bender? Even though our point of reference is the (+) strand, and the RNA polymerase travels from 5' to 3' on the (+) strand, the RNA polymerase actually reads and transcribes from the other strand, the Template Strand. Another name for this is the 'minus' (-) strand. In the next section we're going to see why RNA polymerase does this, along with the 'cipher' that it uses to transcribe DNA sequences into RNA sequences.

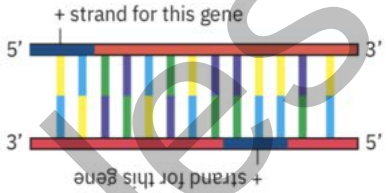


Figure 4-27. The blue segment of each strand represent different non-coding promoters. On the top strand, the RNA polymerase would bind and continue to the right toward the 3' OH end. On the bottom strand, the RNA polymerase would bind to the blue region and transcribe to the left toward the 3' OH end of that strand.

During transcription: The secret cipher for transcribing DNA to RNA

After discovering DNA's structured in the 1950s, the next great mystery was how DNA could hold the information for other molecules such as RNA and proteins. It took decades of research to unravel this mystery.

As you saw in Figure 4-19 and Figure 4-20 RNA nucleotide structures are quite similar to DNA nucleotides. There is one other important difference between DNA and RNA: There is no 'T' thymidine nucleotide in RNA. Instead there is a 'U' nucleotide for uridine, hence, GAUC.

Ordering DNA *Pro-tip*

What DNA strand should you use when ordering DNA? If you see a DNA sequence for sale online, or if you plan to design a DNA sequence to order, ignore the sequence of the template strand. In all instances, euse the DNA sequence of the Leading (+) strande in the order of 5' to 3'. If you know the coding (+) strand sequence, it is easy to determine the template (-) strand. This is simply a convention that the research and biotechnology community has agreed upon.

Check Point!

You've accomplished quite a lot so far on your journey, Congratulations! Below is a 21 point checklist summary of what we learned and did in Chapters 1-5. Review it before going further, and make sure to repeat any exercises or breakouts if you feel it necessary!

LB agar plates are made. They are the food (sugar, amino acids) and substrate (solid agar scafolding in a petri dish) that E. coli can grow on. Non-selective LB agar plates are used to grow "Blank cells", while selective LB agar plates are used to grow genetically engineered cells that have a selection marker.

Cells are collected from a stab, streaked onto a non-selective LB agar plate and incubated at 37 ˚C for 16-24 hours so that fresh, fast-growing non-engineered cells can be used for the genetic engineering experiment.

Chemically competent cells are made in a cold environment by collectiong fast growing E. coli cells and mixing theminto Transformation Buffer. Transformation Buffer is a liquid that contains salts such as calcium chloride so that the cells are better able to take in DNA.

A DNA plasmid is mixed into the chemically competent cells, incubated and then heat shocked at 42 ˚C in order to help the DNA plasmids pass through the cell membrane and cross into the cells. The cells are cooled to trap the DNA inside.

The cells are recovered in Recovery Media at 37˚C to begin their growth cycle, division, and so that they begin expressing their selection marker, such as an antibiotic resistance enzyme, if necessary.

In order to express the selection marker, the DNA plasmid includes a specific gene. This gene sequence has a promoter, RBS, and protein coding sequence (with a start and stop codon). After transcription and translation, the gene results in the expression of a protein enzyme called chloramphenicol acetyltransferase that can break down the antibiotic:

Sigma factors bind to the promoter and recruit the RNA polymerase which creates an initiation sequence. It then tries to escape the promoter

If the RNA polymerase escapes the promoter, it glides down the DNA molecule, unzipping it and, as the correct complementary ribonucleotide enters the polymerase, adds it to the string of RNA

Rho-dependent (Rho proteins) or rho-independent factors (such as terminator hairpins) cause the RNA polymerase to "slip off" the DNA. The RNA polymerase then releases the RNA transcript into the cytoplasm of the E. coli cell - transcription ends.

Initiation factors bumping around the cell bump into the RBS sequence of the RNA transcript and bind to it. They recruit the ribosome which also binds to the RBS and locks in thanks to ribosomal RNA (rRNA).

If a start codon is present, a special starter tRNA (fMet) binds to the ribosome and kicks starts translation. The ribosome rides down the RNA transcript like a train on train tracks and the many aminoacyl tRNAs that are complementary to the particular RNA codon in the RNA transcript allow the ribosome to know what amino acids to add to the growing amino acid string.

The ribosome encounters a Stop Codon where a release factor resides and concludes the translation of the amino acid string. The ribosome falls off the RNA transcript

While translation is happening, as well as afterwards, the amino acid string folds up on itself to form a three-dimensional macromolecule called a protein. It is the three-dimensional structure of the protein that determines its function.

ZERO TO HERO LEARNING JOURNEY

WHAT IS DNA?

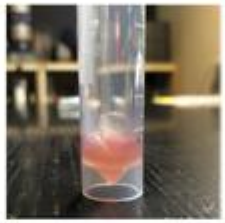
1

HANDS-ON SKILLS

- Suspend cells
- Lyse cells using surfactants
- Precipitate DNA

FUNDAMENTAL CONCEPTS

- Evolution: It is natural for DNA to change
- The road to precisely editing DNA
- DNA extractions in the real world
- Atoms, molecules, and macromolecules (nucleic acid)
- Nucleotides, DNA strands
- Nomenclature of DNA



BIG TAKEAWAY:

DNA is the 'blueprint of living things'. During research and biotech development, DNA is often modified to 'reprogram' living organisms. DNA is at the center of it all; understanding it is a key part of independent research.

MATERIALS:

Zero to Hero Book PME001
DNA extraction Kit
WWG001-S or -G
or your own materials
(Isopropyl alcohol, liquid soap, salt, distilled water, fruit, coffee filter, glasses)

BIOSAFETY & LAB SETUP

2

HANDS-ON SKILLS

- How to experiment safely
- Organizing your space
- Basic supplies such as cleaning & personal protection equipment
- Getting equipment & kits

FUNDAMENTAL CONCEPTS

- The 4 Biosafety levels
- Rules & regulations in biotechnology
- What type of equipment is necessary?
- What is wetware?
- Lab safety & best practices

BIG TAKEAWAY:

Setting up a lab space is straightforward. Doing science safely is simple. Making biosafety a standard part of your routine is an important part of becoming a genetic engineering hero & an independent researcher.

MATERIALS:

Zero to Hero Book PME001
DNA Playground(s)
HWE001-S or -G
Safety set SMG001-G
or your own materials
(Incubator, ice bucket, hot bath, gloves, lab coats/aprons)

GROWING CELLS

3

HANDS-ON SKILLS

- Make LB agar plates
- Streak & grow cells
- Incubate cells
- Inactivate cells
- See and smell bacteria

FUNDAMENTAL CONCEPTS

- Lab *E. coli vs* 'bad' *E. coli* and its history
- Cells as microfactories - a comparison between factories and cells to learn cell structure
- Macromolecules: carbs, lipids, proteins

BIG TAKEAWAY:

Learning what cells are made of and how to grow them is a very important part of studying biology and creating biotechnology, especially for an independent research project.

MATERIALS:

Zero to Hero Book PME001
Canvas Kit
WWA003-S or -G
+ Ch.2 lab setup & safety

ENGINEERING CELLS

4

HANDS-ON SKILLS

- Make competent cells (take in DNA)
- Handle DNA plasmids
- Engineer cells with DNA plasmids (bacterial transformation)
- Differentiate between engineered & non-engineered cells
- Positive & Negative controls

FUNDAMENTAL CONCEPTS

- Basics operating environment of a cell
- How do cells know how to start, do and stop reading DNA (transcription)
- What is a gene?
- DNA plasmids

BIG TAKEAWAY:

Cells can't think! Chemistry is an important part of how cells operate and how to get DNA plasmids into cells. Learning the chemistry of how to manipulate cells is an important part of doing independent research projects.

MATERIALS:

Zero to Hero Book PME001
Engineer-it Kit
WWE004-S or -G or -F
+ Ch.2 lab setup & safety

EXTRACTING PROTEINS

CHAPTER 5

HANDS-ON SKILLS

- Amplifying cultures
- Collect & Lyse cells to free engineered molecules
- Centrifugation & pelleting
- Sterilization with nanofiltration

FUNDAMENTAL CONCEPTS

- How do cells know how to start, do and stop reading RNA (translation)
- Proteins & enzymes
- Promoters vs ribosomal binding site
- The other RNAs (rRNA & tRNA)
- The RNA to protein cipher

BIG TAKEAWAY:

Upon engineering cells with a DNA plasmid, cells read the DNA and transcribe RNA, then read that RNA to translate into proteins. Proteins are sometimes the desired end-product and extracting them from cells is an important step to doing independent research.

MATERIALS:

Zero to Hero Book PME001
Engineer-it Kit
WWE004-S or -G or -F
Plate Extract-it Kit
WWG005-S or -G
Microcentrifuge
HWE003 or similar
+ Ch.2 lab setup & safety

ENZYME PROCESSING

CHAPTER 6

HANDS-ON SKILLS

- Processing small molecules to create products (smell or color) using an enzyme.

FUNDAMENTAL CONCEPTS

- Basics of enzymatic chemical reactions
- The Four B's of cell function in action
- Protein enzymes as catalysis in cells
- Basic structure of atoms
- The four major types of bonds
- Metabolic pathways

BIG TAKEAWAY:

Engineering cells is often done to get a protein enzyme used in a chemical reaction with its end-product being what is desired. The engineered protein is a means to an end! Understanding enzymes and enzymatic reactions are key to success in life science research.

MATERIALS:

Zero to Hero Book PME001
Smell-it Kit WWE006-S or -G
or Blue-it Kit WWE007-S or -G
Microcentrifuge
HWE003 or similar
+ Ch.2 lab setup & safety

MANUALLY TURNING-ON GENES

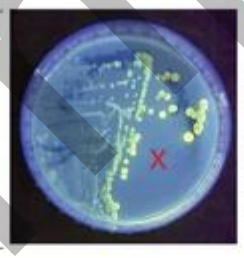
CHAPTER 7

HANDS-ON SKILLS

- Induce gene expression using chemicals, heat or light.

FUNDAMENTAL CONCEPTS

- Mechanisms of gene expression
- Reinforce the coding and non-coding regions of genes learned in Ch. 4-5.
- Setting the stage for designing custom plasmids.



BIG TAKEAWAY:

Gene regulation is a broad topic and an important part of life science research and projects. Learning gene regulation with hands-on examples is key to expanding the independent researcher's imagination & 'toolbox'.

MATERIALS:

Zero to Hero Book PME001
Induce-it Kit WWE008-S or -G
or Heat-it Kit WWE009-S or -G
or RGB Kit WWA010-S or -G +
(HWA010-S or HWE001-G)
+ Ch.2 lab setup & safety

GOING FURTHER: INDEPENDENT PROJECTS

CHAPTER

SCIENCE RESEARCH AT YOUR OWN PACE

- Minilabs, Junior R&D Kits are used for independent research using the methods from Chapters 1-7
- Junior R&D kit is a 'blank' engineering kit with custom antibiotics to be used with a project plasmid.
- Use the BioExplorer to learn liquid culturing and do biomanufacturing with real-time sensing.
- Partner with Amino Labs to turn a research project into an experiment kit and earn revenue!

MATERIALS:

Junior R&D Kit
WWE014
Microcentrifuge
HWE003 or similar
BioExplorer
HWE002-S (optional)
+ Ch.2 lab setup & safety