November 2022

Differentiated ABE

By Anna-Maree Wells, ABE Australia

ABE Master Teacher Fellowship Program

AMGEN® Biotech Experience

Scientific Discovery for the Classroom

The curriculum projects designed by the 2021–22 ABE Master Teacher Fellows are a compilation of curricula and materials that are aligned with Amgen Biotech Experience (ABE) and prepare students further in their biotechnology education. These projects were created over the course of a 1-year Fellowship in an area of each Fellow's own interest. Each is unique and can be adapted to fit the needs of your individual classroom. Objectives and goals are provided, along with expected outcomes. Projects can be used in conjunction with your current ABE curriculum or as an extension.

As a condition of the Fellowship, these classroom resources may be downloaded and used by other teachers for free. The projects are not edited or revised by the ABE Program Office (for content, clarity, or language) except to ensure safety protocols have been clearly included where appropriate. We are grateful to the ABE Master Teacher Fellows for sharing their work with the ABE community.

If you have questions about any of the curriculum pieces, please reach out to us at ABEInfo@edc.org, and we will be happy to connect you with the author and provide any assistance needed.

AMGEN® Biotech Experience

Scientific Discovery for the Classroom

Australia

ABE Master Teacher Fellowship

Differentiated ABE

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PROGRAM SITE: Australia

SUMMARY OF PROJECT IDEA

Differentiate ABE Australia Student and Teacher Guides to align with the Stage 6 Investigating Science Syllabus

Estimated Project Duration: (# weeks/class periods)

2 weeks

Student Understandings/Big Ideas:

How have developments in technology led to advances in scientific theories and laws that in turn drive the need for further developments in technology?

Student Understandings/Learning Outcomes:

Assess the impact that developments in scientific theories, laws, and models have had on the development of new technologies including impact the discovery of DNA has had on the development of biotechnologies to genetically modify organisms.

Assessments:	Standards*:
Pre-Assessment Quiz: How does the DNA code work? **Formative Assessment:** Biotechnology timeline **Summative Extended Response:** Assess the impact the discovery of DNA has had on the development of biotechnologies to genetically modify organisms	• INS12-1 • INS12-2 • INS12-4 • INS12-13 Source: New South Wales Stage 6 Investigating Science syllabus

Opening "Hook":

Students will be challenged to genetically modify an organism.

Prior Knowledge and Skills:

Recall:

- The structure of DNA
- How the structure of DNA was discovered
- The role DNA plays in living organisms

Lab safety skills

Cultural Relevancy and Personal Connections:

- Who knows someone impacted by diabetes, cancer, heart disease or a genetic disorder?
- How are vaccines made to prevent infectious diseases?
- Who knows a farmer impacted by drought/pests and disease?
- How many people worldwide are affected by food shortage/famine?

Learning Activities at a Glance:

Activity: How does DNA code for living organisms?	Materials and Resources Needed: • YouTube Clip https://www.youtube.com/watch?v=YovwL0LwkUg • Blooket Game

Learning Activities at a Glance:	
	https://dashboard.blooket.com/edit?id=627f5cfbe0d5145bb52d89e9 • Understanding how DNA works worksheet
Activity: Comparing biotechnologies before and after the discovery of DNA structure	Materials and Resources Needed: • Biotechnology Timeline • Venn Diagram worksheet
Activity: Tools of the trade: Micropipetting	Materials and Resources Needed: • AMGEN KIT • Differentiated student guide
Activity: Tools of the trade: Gel electrophoresis	Materials and Resources Needed: • AMGEN KIT • Differentiated student guide
Activity: Tools of the trade: Plasmids and restriction enzymes	Materials and Resources Needed: • AMGEN KIT • Differentiated student guide
Activity: Transforming bacteria	Materials and Resources Needed: • AMGEN KIT • Differentiated student guide
Activity: Colony PCR	Materials and Resources Needed: • AMGEN KIT • Differentiated student guide
Activity: Applications of biotechnologies to genetically modify organisms	Materials and Resources Needed: • LabXchange Pathway: Investigating Science Biotechnology Depth Study https://www.labxchange.org/library/pathway/lx-pathway:adfaa302-f85d-4e0c-bd69-55e49600e298 • Applications of Biotechnologies Note making Scaffold
Activity: Summative extended response	Materials and Resources Needed: • Extended Response Scaffold and marking guideline

How does DNA code for living organisms?

1. **Overview:** This lesson outlines the central dogma of biology and provides an introductory explanation of the flow of genetic information in organisms for students who have not previously studied the senior Biology course.
2. **Learning Goals:**
   - Recall the structure of DNA
   - Outline how DNA codes for RNA and RNA codes for proteins
   - Identify those proteins that are responsible for organisms' traits
3. **Assessed Outcome:** INS12-4
4. **Key Vocabulary:** DNA, RNA, ribosome, nucleus, protein, amino acid, traits
5. **Materials and LabXchange Pathway(s):**
   - YouTube Clip https://www.youtube.com/watch?v=YovwL0LwkUg
   - Blooket Digital Game: https://dashboard.blooket.com/edit?id=627f5cfbe0d5145bb52d89e9
   - Understanding How DNA Works worksheet
6. **Teacher Preparation:** Students will need access to a digital device and a copy of the *Understanding how DNA works* worksheet. Teacher will need to access to smartboard or MLD to present the Blooket Game
7. **Lab Safety Considerations:** Nil
8. **Sequence**

Activity Description	*Time*	*Materials*
1. Watch and discuss YouTube Clip: What is DNA and how does it work?	10 mins	https://www.youtube.com/watch?v=YovwL0LwkUg
2. Play digital Blooket game to consolidate key ideas.	20 mins	https://dashboard.blooket.com/edit?id=627f5cfbe0d5145bb52d89e9
3. Formative Assessment: Students annotate and explain diagrams to demonstrate their understanding of the flow of genetic information in organisms. Teacher clarifies any misconceptions.	30 min	Understanding how DNA works worksheet

Comparing biotechnologies before and after the discovery of DNA structure

1. **Overview:** This is a whole class activity in which students gain an insight into the history of biotechnology and an understanding of the important role understanding the structure of DNA has had on the explosion of modern biotechnologies to genetically modify organisms.

2. **Learning Goals:**

   - Identify that human shave been genetically modifying organisms for thousands of years
   - Compare features of biotechnologies before and after the structure of DNA was described
   - Discuss the importance of understanding the structure of DNA to developing biotechnologies to genetically modify organisms

3. **Assessed Outcome:** INS12-4, INS12-13

4. **Key Vocabulary:** biotechnologies, genetically modified organisms

5. **Materials and LabXchange Pathway(s):**

   - Biotechnology Timeline
   - Venn Diagram worksheet

6. **Teacher Preparation:** copies of worksheets

7. **Lab Safety Considerations:** Nil

8. **Sequence**

Activity Description	*Time*	*Materials*
1. Class discussion about key events in the history of biotechnologies; students identify where on the timeline the discovery of the structure of DNA is located.	20 min	Biotechnology Timeline
2. Class compares similarities and differences between the biotechnologies developed before and after the discovery of the structure of DNA.	10 min	• Biotechnology Timeline • Venn Diagram

Applications of biotechnologies to genetically modify organisms

1. **Overview:** Students research more deeply three examples of biotechnologies to genetically modify organisms, outline the problem solved by the biotechnology technique, and explain the importance of knowing the structure of DNA to the development of the biotechnology technique.
2. **Learning Goals:** Understand three additional examples of biotechnologies that genetically modify organisms
3. **Assessed Outcome:** INS12-1, INS12-4, INS12-13
4. **Key Vocabulary:** biotechnology, genetically modified organisms
5. **Materials and LabXchange Pathway(s):** Investigating Science Depth Study https://www.labxchange.org/library/pathway/lx-pathway:adfaa302-f85d-4e0c-bd69-55e49600e298
6. **Teacher Preparation:** Students will need access to digital devices for research and a copy of the note making scaffold
7. **Lab Safety Considerations:** Nil
8. **Sequence**

Activity Description	*Time*	*Materials*
Students research three examples of biotechnologies used to genetically modify organisms.	60 min	LabXchange Pathway: Investigating Science Depth Study

Extended response summative assessment

1. **Overview:** Students apply their learning from all previous learning activities to plan, write and self/peer assess an extended written response
2. **Learning Goals:**
    - Demonstrate understanding of the importance of the discovery of DNA on technologies to genetically modify organisms
    - Plan and write an extended response
    - Use a marking guideline to self/peer assess and provide warm and cold feedback
3. **Assessed Outcome:** INS12-1, INS12-4, INS12-13
4. **Key Vocabulary:** biotechnology, genetically modified organisms
5. **Materials and LabXchange Pathway(s):** LabXchange Pathway: Extended response scaffold and marking guideline
6. **Teacher Preparation:** Students will need access to digital devices for research and a copy of the extended response scaffold and marking guideline.
7. **Lab Safety Considerations:** Nil
8. **Sequence**

Activity Description	*Time*	*Materials*
Students research three examples of biotechnologies used to genetically modify organisms.	60 min	Extended response scaffold and marking guideline

AMGEN BIOTECHNOLOGY EXPERIENCE

MOUNT VIEW HIGH SCHOOL

YEAR 12 INVESTIGATING SCIENCE DEPTH STUDY

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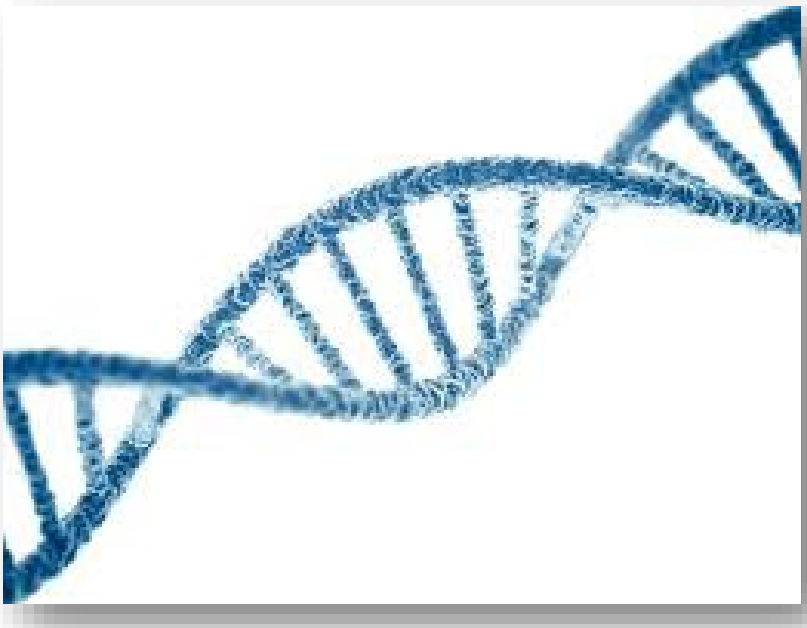


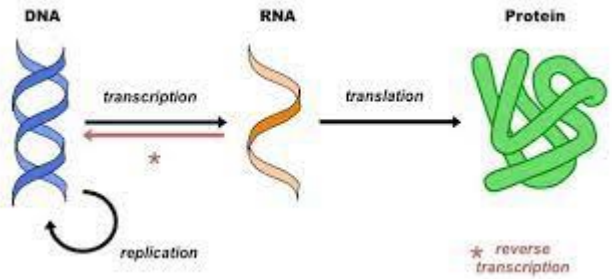
Assess the impact of the discovery and understandings of the structure of DNA on the development of biotechnologies to genetically modify organisms.

Contents

THE CENTRAL DOGMA OF BIOLOGY – How DNA codes for life

The discovery in 1953 of the double helix, the twisted-ladder structure of deoxyribonucleic acid (DNA), by James Watson and Francis Crick marked a milestone in the history of science and gave rise to modern molecular biology, which is largely concerned with understanding how genes control the chemical processes within cells. In short order, their discovery yielded ground-breaking insights into the genetic code and protein synthesis. During the 1970s and 1980s, it helped to produce new and powerful biotechnologies, specifically recombinant DNA research, genetic engineering, rapid gene sequencing, and monoclonal antibodies, techniques on which today's multi-billion-dollar biotechnology industries in both medicine and agriculture are founded. Major advances in science, namely genetic fingerprinting and modern forensics, the mapping of the human genome, and the emerging fields of gene therapy and xenogeneic transplants, all have their origins in Watson and Crick's inspired work.

Following the discovery of the structure of DNA, Francis Crick proposed that DNA led to the formation of RNA, which in turn, led to the synthesis of proteins. Experimental evidence supported his proposal. The 'flow of genetic information' became known as the 'central dogma of molecular biology'. In 1961, Francis Crick and Sydney Brenner provided the missing link to decoding DNA, with their discovery that genes use three-letter 'words' or triplets of bases called codons to code instructions for each amino acid in a protein chain.



Scientists already knew that polypeptides were chains of amino acids and that these polypeptides joined to form proteins. It took about 5 more years to reveal specifically which triplet coded for which amino acid. In 1968, Marshall Nirenberg received a Nobel Prize for his work cracking the genetic code for protein synthesis, listing the 60 triplets that code for each of the 20 amino acids in proteins.

Learning Activity 1: How does DNA code for living organisms

Learning Goals:

- Recall the structure of DNA
- Outline how DNA codes for RNA and RNA codes for proteins
- Identify those proteins are responsible for organisms' traits

1. Watch the following animation and play Blooket with your class to consolidate your understanding the of *Central Dogma of Biology.*

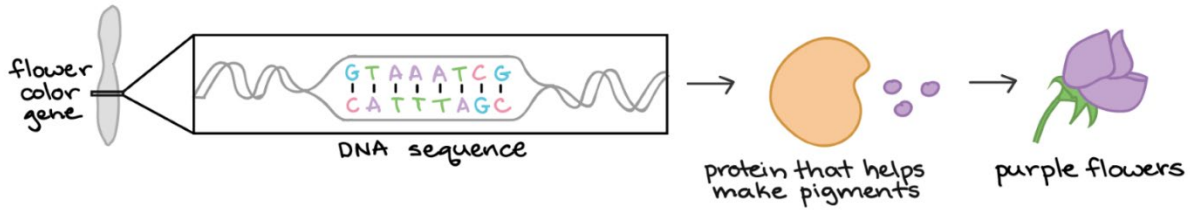
YouTube Clip https://www.youtube.com/watch?v=YovwL0LwkUg

Blooket Game https://dashboard.blooket.com/edit?id=627f5cfbe0d5145bb52d89e9

2. Applying your understanding of how DNA works.

The diagram below summarises the process of how DNA codes for a particular trait observed in an organism.

A. To test your understanding of this process, annotate and explain **where** each step occurs in the cell and **what** is happening in this diagram. Try and explain how DNA codes for traits in living things to another student or family member.



B. Scientist have discovered they can produce red flowers by changing the thymine base to an adenine base in the first codon of the DNA sequence that makes the pigment. Annotate the diagram below to show how this is possible.

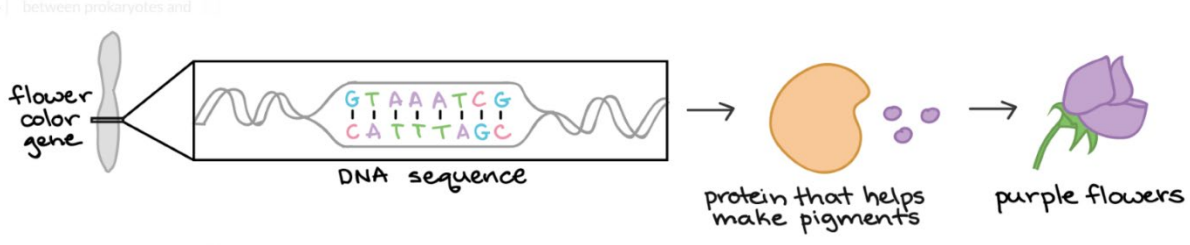


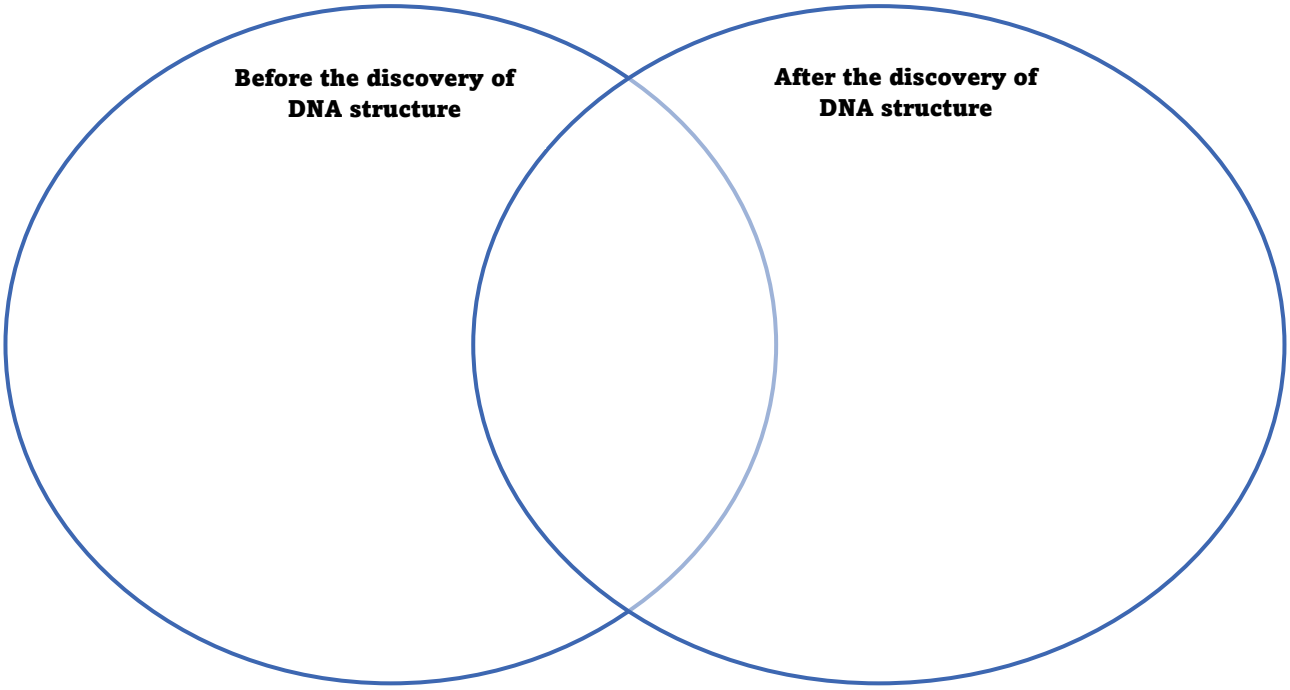
Diagram from Kahn Academy https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/translation/a/intro-to-gene-expression-central-dogma

Learning Activity 2: Comparing biotechnologies before and after the discovery of DNA structure

Learning Goals:

- Identify that human have been genetically modifying organism for thousands of years
- Compare features of biotechnologies before and after the structure of DNA was described
- Discuss the importance of understanding the structure of DNA to developing biotechnologies to genetically modify organisms

1. Using the **Biotechnology Timeline** provided at the back of your booklet complete the Venn diagram below to compare the biotechnologies before and after the discovery of the structure of DNA.



Treating disease with gene cloning

Until relatively recently, people with certain diseases had to rely on remedies that were expensive and sometimes difficult to obtain. Many of these diseases are the result of the loss of a single protein function, either because the protein produced is defective or because it is not produced in normal amounts (a protein is a large biomolecule that carries out essential functions in cells). For example, individuals with haemophilia, a bleeding disorder in which blood fails to clot normally, make little or no clotting factor protein; a deficiency of human growth hormone can cause poor growth, delayed puberty, and muscle weakness in children, and fatigue, reduced muscle and bone mass, baldness, increased body fat and memory loss in adults.

By providing the patient with a functional protein, the symptoms of these diseases can be alleviated. Before genetic engineering technology, these therapeutic proteins had to be extracted from natural sources such as human blood or animal tissue, a process that was generally difficult, inefficient, and expensive. Pharmaceutical companies can use genetic engineering—or gene cloning, as it is often called—to make these proteins cost-effectively, in far greater quantities, without the impurities and viruses that can be transmitted from blood and tissue samples. Gene cloning involves inserting the

human gene that encodes the protein into bacteria where the protein is made along with all the other bacterial proteins.

CONSIDER: What do you already know about cloning?

The ability to make enough of the proteins to treat diseases is the result of two key discoveries about bacteria made by scientists in the 1970s and '80s. The first discovery was that bacteria contain tiny circles of DNA, called **plasmids**, which sometimes contain genes that can make them resistant to antibiotics. The second discovery was that bacteria also contain proteins called **restriction enzymes** that can cut DNA at very specific places.

The findings made by basic research often led to fundamental understandings about the nature of life. In some instances, these findings can also lead to new technologies that can improve life. With the discovery of plasmids and restriction enzymes, a whole new era of genetic engineering was launched. Scientists now can generate products that can improve health in ways never imagined.

One of the first pharmaceutical products produced using these tools was insulin, which is used to treat diabetes, a debilitating and sometimes fatal disease. To generate large quantities of human insulin, the sequences of DNA that contain the codes of human insulin are inserted into a plasmid that is introduced into the common intestinal bacterium *Escherichia coli* (*E. coli*), where the new protein is synthesised along with all the other bacterial proteins. The genetically modified bacteria are then grown in large batches, and the insulin is purified for use in the treatment of diabetes.

CONSIDER: Do you think that treating diabetes with insulin can be considered a cure? What is the difference between a treatment and a cure?

Treating diabetes

Individuals with type 1 diabetes can regulate their sugar levels by monitoring their blood and injecting insulin as needed. Those with type 2 diabetes can sometimes regulate their blood sugar levels by changing their diet and increasing the amount that they exercise. However, in many cases, medications that reduce insulin resistance in cells and increase the levels of insulin in the blood are required to maintain normal blood sugar levels.

Currently, there is no cure for either type of diabetes.

E. coli with a human gene

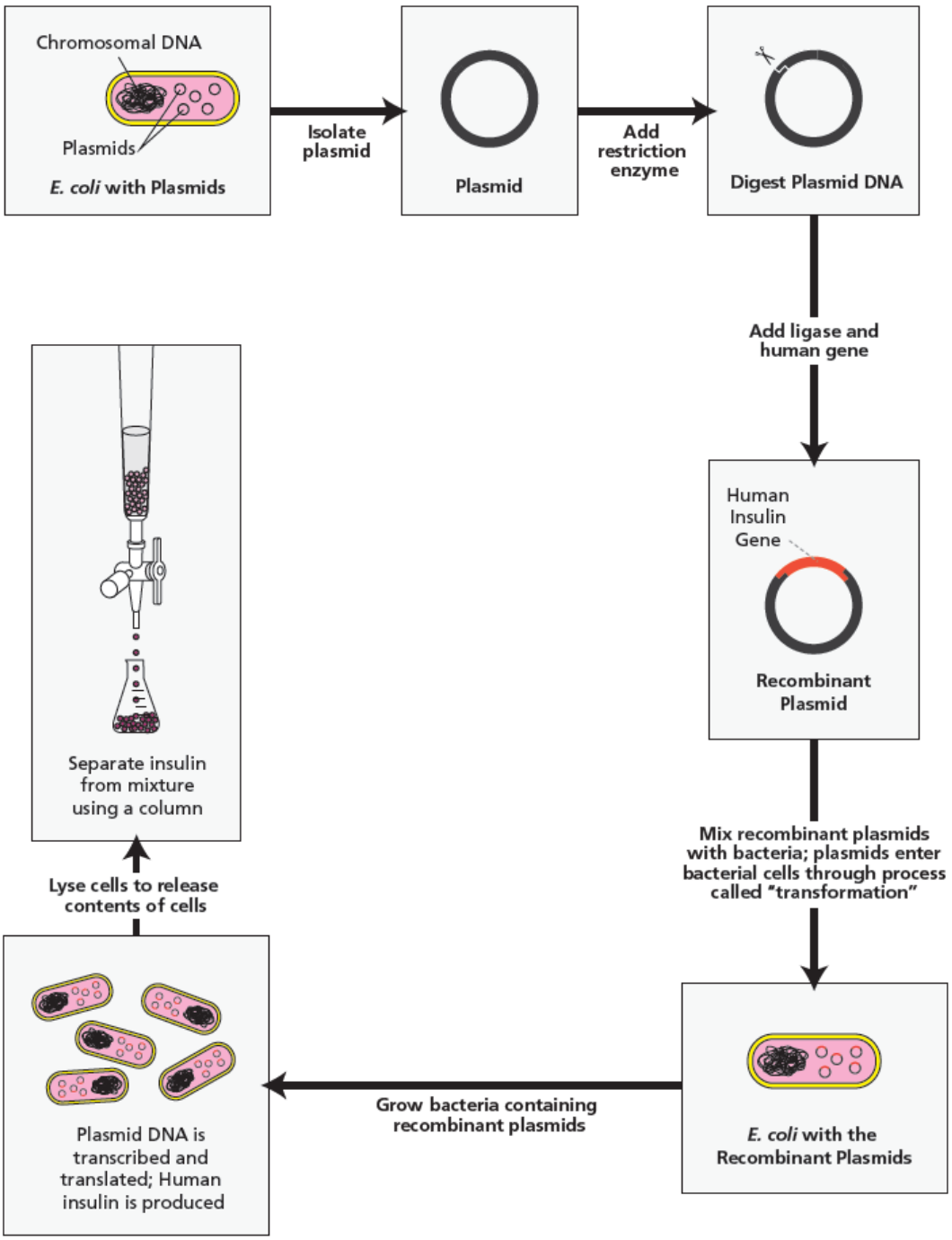
With the rise in diabetes in the population, the need for insulin for treatment is also on the rise. Originally isolated from the pancreases of pigs and cows, most of the insulin used today is genetically engineered human insulin, manufactured by bacteria. DNA sequences encoding human insulin in plasmids are taken up by bacteria, which make the hormone along with all its bacterial proteins. Insulin is then isolated from the bacteria. In 1982, human insulin was the first commercially successful product made by recombinant DNA technology (*recombinant DNA* refers to DNA that contains sequences or genes from two or more sources).

CONSIDER: Why might diabetes be on the rise, especially in teenagers?

Making new proteins in bacteria

Figure P.4 shows how a human protein—in this case, insulin—can be made in bacteria. The insulin is then purified so that it can be used by people with diabetes.

Figure P.4: Making insulin in bacteria



Your Challenge

Your challenge in the Amgen Biotech Experience is to successfully carry out the steps of the genetic engineering process that are used to make insulin and other genetically engineered products. You will learn and practise the techniques and procedures that are part of this process. If you carry out all the steps in the program, you will create your own genetically modified bacteria.

Instead of cloning insulin or another human gene, you will work with a gene from a sea anemone, a soft-bodied animal related to coral and jellyfish (the gene is called *rfp* and the protein made by this gene is called red fluorescent protein). How will you know if you are successful? The bacteria you create will have a new and highly visible trait: They will now produce red fluorescent protein!

DID YOU KNOW?

Red Fluorescent Protein in Sea Anemones

Red fluorescent protein is derived from a protein found in sea anemones. While sea anemones are sedentary, remaining attached to rocks, they are also predatory animals, using their stinging tentacles to catch their prey. The protein glows because it can absorb one colour of light and then emit light of a different color—a process known as *fluorescence*. But why is it important for sea anemones to fluoresce? Our best guess is that fluorescent proteins somehow help sea anemones survive, but the role these proteins play is not yet well understood. Fluorescent molecules may serve as a sunblock, turning harmful UV light into light that is less damaging to the anemone's tissues. Another possibility is that while humans can't detect the fluorescence in bright sunlight, some animals may be able to, causing prey to be attracted to the glow.

Program Introduction: Glossary

Biomolecule: A molecule produced by living cells. Examples include proteins, carbohydrates, lipids and nucleic acids.

Cells: The basic units of any living organism that carry on the biochemical processes of life.

Diabetes: A disease that occurs when the body doesn't produce or properly use insulin.

DNA (deoxyribonucleic acid): A double-stranded biomolecule that encodes genetic information.

Escherichia coli (E. coli): ***E. coli*** is a common bacterium found in the gut of warm-blooded animals. Most strains are harmless, including the strain used in these lab protocols.

Fluorescence: The production of light by a molecule (e.g., red fluorescent protein will release red light when exposed to ultraviolet light).

Gene cloning: Using genetic engineering techniques to create exact copies, or clones, of a gene or DNA sequence of interest.

Genetic engineering: A branch of biotechnology that uses specific procedures and techniques to change an organism's DNA.

Glucose: A sugar that is a major source of energy and biomolecules to sustain life processes. Glucose is absorbed through the intestine and travels in the blood to cells, where it is transported through the cell membrane to be used as energy, to synthesise cells and tissues, and to carry out other essential processes.

Haemophilia: A disease that occurs when the ability of blood to clot is reduced due to lack of one or more blood clotting factors.

Insulin: A hormone produced in the pancreas that controls the amount of glucose in the blood. Insulin is a protein.

Plasmid: A circular molecule of DNA.

Protein: A large biomolecule. Proteins carry out essential functions in cells, from forming cellular structures to enabling chemical reactions to take place.

Receptor: A protein that receives signals from outside the cell. When a signal substance binds to a receptor, it directs the cell to do something, for example to allow biomolecules to enter the cell.

Recombinant DNA: DNA that contains sequences or genes from two or more sources.

Restriction enzyme: A protein that can cut DNA at a specific sequence.

SOME TOOLS OF THE TRADE: Pipetting & Gel Electrophoresis

Learning Goals:

- Use micropipettes and the technique of gel electrophoresis correctly.
- Explain the importance of micropipettes and gel electrophoresis in genetic engineering.
- Assess the accuracy of micropipettes.
- Describe how gel electrophoresis separates DNA.
- Explain how genetic engineering can be used to treat some genetic diseases.

Some tools of the trade: Pipetting & Gel Electrophoresis: Introduction

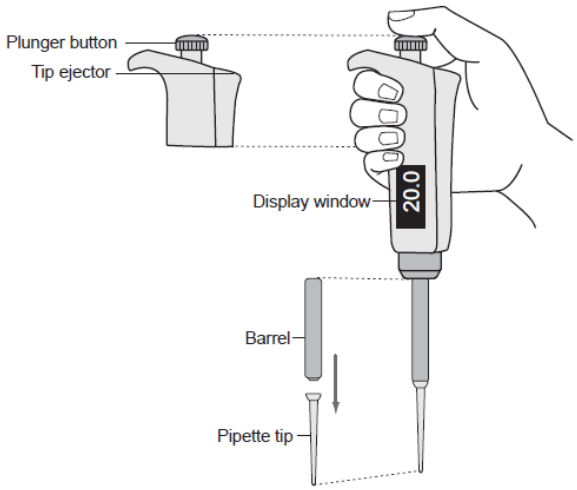
1. Why do you think it is necessary to use very small and exact volumes of reagents in biotechnology?

2. Adding human DNA to bacteria makes it possible to make human insulin. What do you already know about DNA? Be as detailed as possible and discuss the location of DNA in the cell, the DNA structure, the replication of DNA, and the components of DNA.

How to use a micropipette

The purpose of this laboratory is to introduce you to an important tool used in genetic engineering: the micropipette, shown in **Figure 1.1**. A micropipette is used to transfer very small and exact volumes of liquids in either millilitres (mL, thousandths of a litre) or microliters (µL, millionths of a litre), which are the measurements of volume most often used in genetic engineering.

Figure 1.1: A P20 micropipette



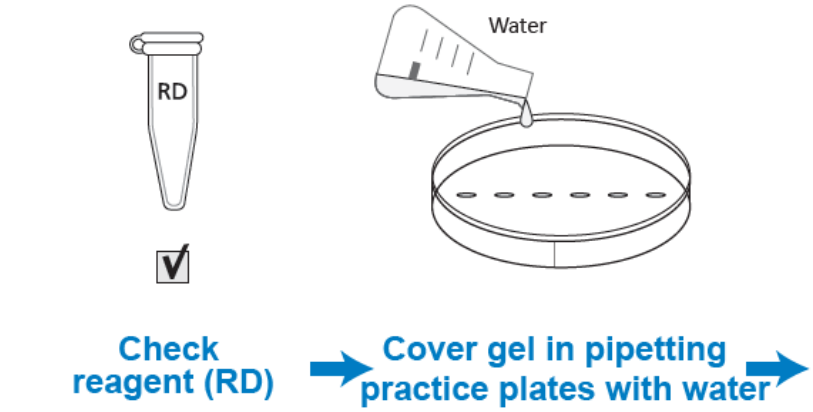
Lab technique:

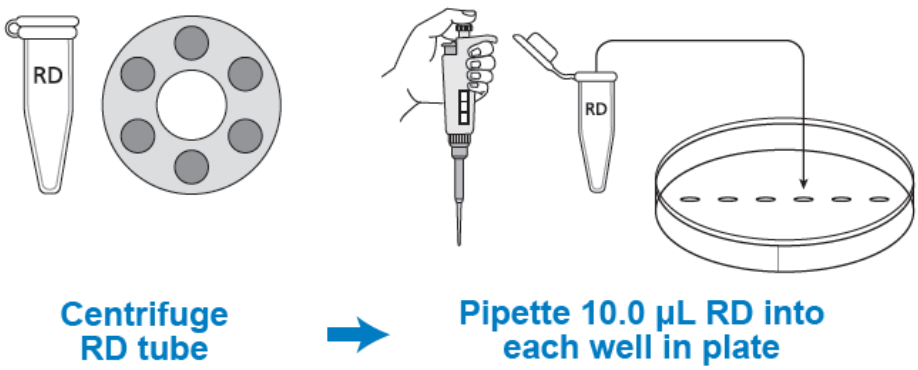
- Hold the tube at eye level so you can see if the solution is loaded and dispensed properly.
- After placing the tip on the micropipette, gently twist the top of the tip to ensure that it is firmly attached to the barrel.
- When loading the pipette, always press the plunger to the first stop position BEFORE immersing the tip in the liquid. This will prevent an air bubble being expelled into the liquid.
- Never set the P200 micropipette lower than 20 µL or higher than 200 µL. Doing so could damage the equipment.
- Do not lay down a micropipette with fluid in the tip or hold it with the tip pointed upward.
- Be careful not to place your pipette tip into the well or you might puncture the gel, which will make the well unusable.

Further Information:

About Gel Electrophoresis can be found in the LabXchange Pathway: Tools and Techniques in Biotechnology: Micropipetting: https://www.labxchange.org/library/pathway/lx-pathway:2cd1b2af-481a-4b2e-ad51-5904c2ec16fe

Some tools of the trade: Pipetting: Flow Chart





Some tools of the trade: Methods

You will practise pipetting RD into preformed wells in an agarose gel.

1. Check your rack to make sure that you have the RD tube.
2. Fill the pipetting practice plate with water to a level that just covers the entire surface of the gel. If you see any “dimples” over the wells, add more water.
3. Set the P20 micropipette to 10.0 µL and put on a pipette tip.
4. Load the pipette with 10.0 µL of RD.
5. Dispense RD into a well in the practice plate by doing the following:
    a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
    b. Lower the pipette tip until it is under the buffer but just above the well.
    c. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.
    d. While the plunger is still depressed, pull the tip out of the buffer so that you don’t aspirate the sample or buffer.
6. Repeat steps 4 and 5 until all the practice plate wells have been filled.
7. Eject the pipette tip.

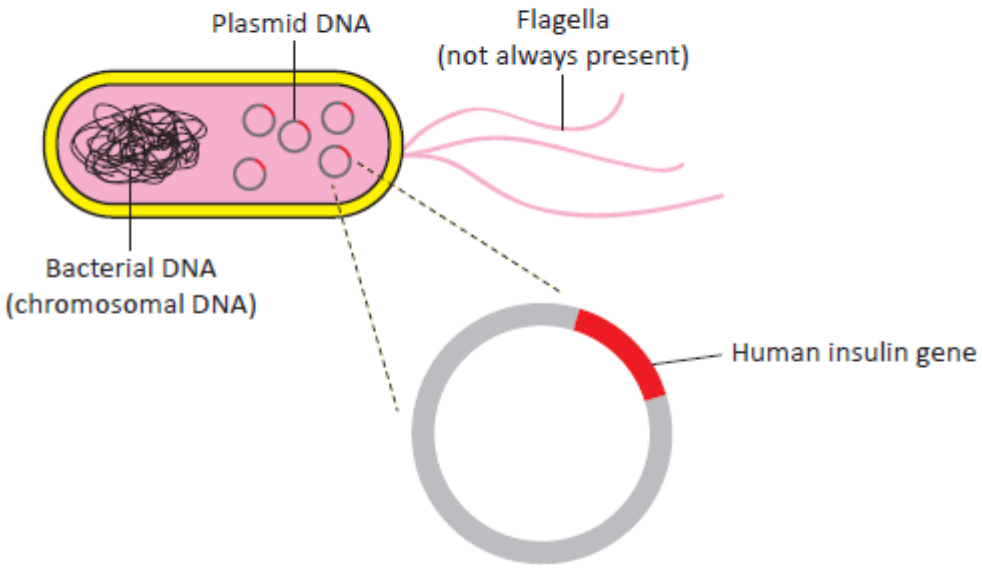
THE GENETIC ENGINEERING PROCESS

Do you know somebody who takes *insulin*, or a *blood clotting factor*, or *human growth hormone*? These substances are all *proteins* manufactured in certain human cells. If those cells fail to make these proteins, the diseases *diabetes*, *haemophilia*, and *growth deficiency* can result. A patient with one of these diseases must be treated with the missing protein.

CONSIDER: Prior to genetic engineering, how could people get missing proteins for a genetic disease?

Before the development of genetic engineering, it was difficult to obtain human proteins to treat people who needed them. Now, bacteria can make these proteins because scientists have figured out a way to change bacterial DNA by adding human DNA (see **Figure 1.4**).

Figure 1.4: Bacterial cell with human DNA



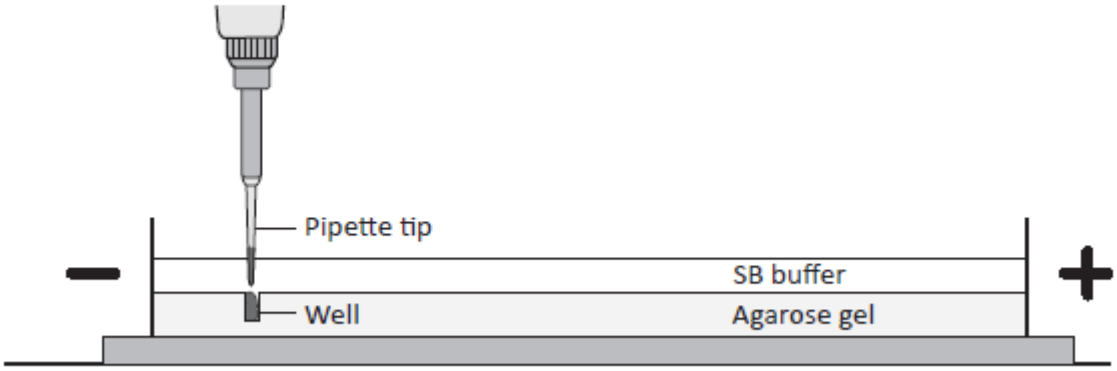
What is the relationship between DNA and proteins? Both are biomolecules—large molecules made by living cells. When scientists investigated traits in organisms, they found that proteins were responsible for traits. For example, consider a plant that has the trait of red flowers. The flowers' red pigment is produced by the action of an enzyme (one kind of protein). The DNA in that plant contains instructions for making proteins, including that enzyme. The part of a DNA molecule that has the instructions for making a particular protein is called a *gene*.

In the genetic engineering process, a human gene is added to a *plasmid*, a small circular piece of DNA found in many bacteria. The plasmid is taken up by bacterial cells, and the cells make the human protein that is encoded by the human gene along with their own proteins. During this process, biotechnologists use a combination of tools, some human-made and some biological.

The purpose of this laboratory is to give you experience with gel electrophoresis, which is used to separate and identify a mixture of biomolecules including DNA; the components of each mixture can then be identified by their location in the gel. Gel electrophoresis works because biomolecules have a negative charge, which means that they will move in response to an electric charge. The biomolecules move through a gel, and their speed varies primarily according to their weight, although molecular shape and degree of charge also influence their movement. In the genetic engineering process, gel electrophoresis is used to separate and identify plasmids and short linear pieces of DNA.

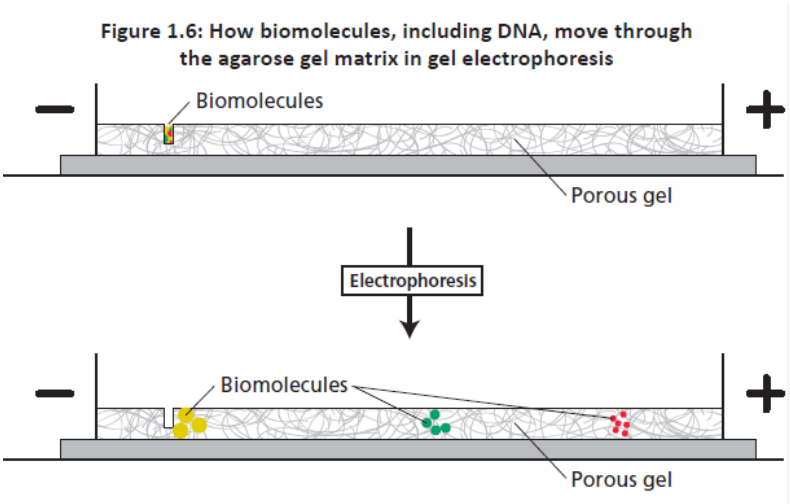
The electrophoresis set-up consists of a box containing an agarose gel and two electrodes that create an electric field across the gel when the box is attached to a power supply. The negative electrode is black, and the positive electrode is red. Samples of biomolecules are pipetted into wells near the negative (black) electrode. The samples move through the gel toward the positive (red) electrode, as shown in **Figure 1.5**.

Figure 1.5: The gel electrophoresis unit



The gel that the biomolecules move through is composed of *agarose*, a polysaccharide (complex sugar) found in seaweed. Its structure is a porous matrix (like a sponge) with lots of holes through which the solution and biomolecules flow. See **Figure 1.6**.

Figure 1.6: How biomolecules, including DNA, move through the agarose gel matrix in gel electrophoresis



Consider: In what circumstances might it be important to use gel electrophoresis to separate and identify plasmids and short linear pieces of DNA?

Further Information:

About Gel Electrophoresis can be found in the LabXchange Pathway: Tools and Techniques in Biotechnology: Gel Electrophoresis: https://www.labxchange.org/library/pathway/lx-pathway:33b08759-5d13-4128-8867-68428a8d1081

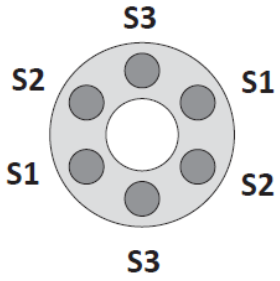
Part A: Separating Dyes with Gel Electrophoresis

Now you will use gel electrophoresis to separate different dyes. First you will add dyes into wells in the gel electrophoresis unit. You will then turn the unit on to move the negatively charged dyes through the gel. (You will share the electrophoresis boxes with one other group; your teacher will tell you which wells your group should use.)

1. Check your rack to make sure that you have the three dye solutions (S1, S2, and S3).
2. Review **Figure 1.5**. Check to make sure that the wells in the gel are located near the negative electrode.
3. Fill the box with 1x SB to a level that just covers the entire surface of the gel. If you see any "dimples" over the wells, add more buffer.
4. Centrifuge the S1, S2, and S3 tubes.

LAB TECHNIQUE:

- ✓ Distribute the tubes evenly in the microcentrifuge so that their weight is balanced.

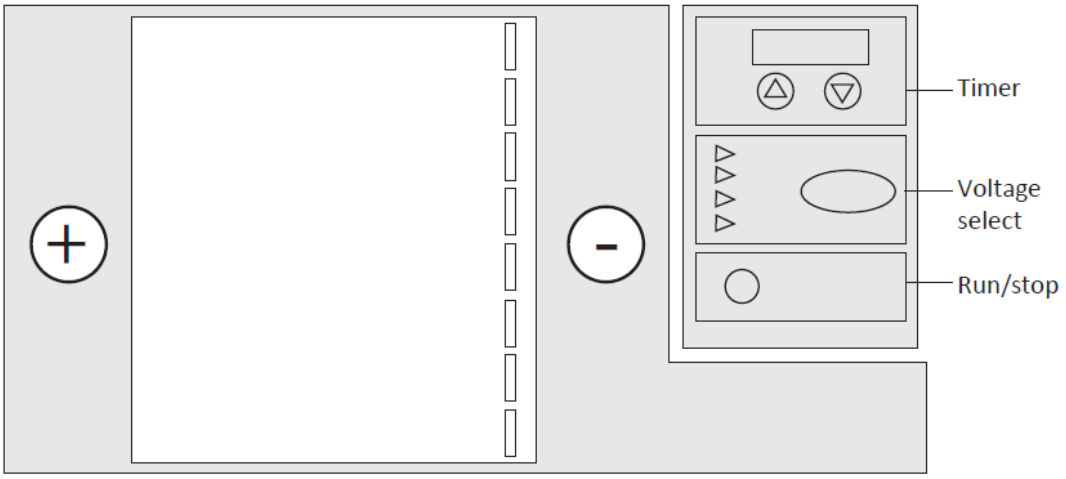


- ✓
- ✓ Do not puncture the gel or it will become unusable.
- ✓ While the plunger is still depressed, pull the tip out of the buffer so that you don't aspirate the solution or buffer.
- ✓ Use a fresh pipette tip for each sample.

5. Make a drawing in your notebook that shows the location of the wells in the electrophoresis box. Record which solution you will place in each well.
6. Set the P20 micropipette to 10.0 µL and put on a pipette tip.
7. Load 10.0 µL of S1 into the pipette.
8. Dispense the S1 into the well you've designated for that solution by doing the following:
    a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
    b. Lower the pipette tip until it is under the buffer but just above the well.
    c. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.

9. Repeat steps 7 and 8 for S2 and S3, using a new pipette tip with each solution.
10. When all the samples have been loaded, close the cover tightly over the electrophoresis box. Close the cover carefully, so that samples don't spill.
11. Plug in the power supply cord on the electrophoresis box. Set the voltage to 100V. Set the timer to 10 minutes. Press the Run/Stop button once to start the electrophoresis. See **Figure 1.7**.

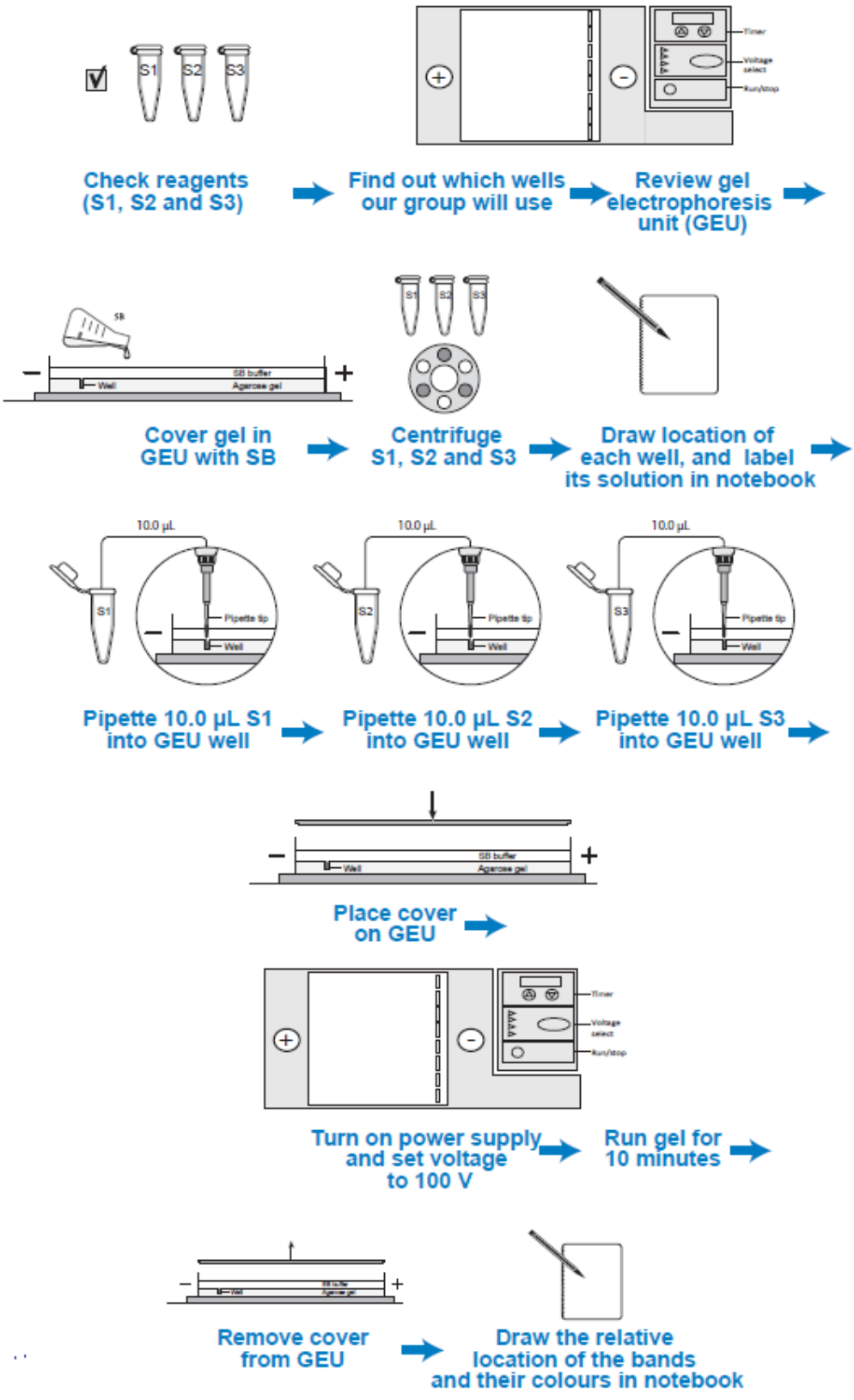
Figure 1.7: Electrophoresis



12. After two or three minutes, check to see if the dyes are moving toward the positive electrode. You should begin to see the purple dye (bromophenol blue) beginning to separate from the blue dye (xylene cyanole).
13. In 10 minutes, or when you can distinguish all three dyes, stop the electrophoresis and unplug the power supply.
14. Carefully remove the cover from the gel box and observe the dyes in the gel.
15. In your notebook, draw the relative location of the bands and their colours in each of the lanes containing your samples.
16. Leave the gel in the gel box.

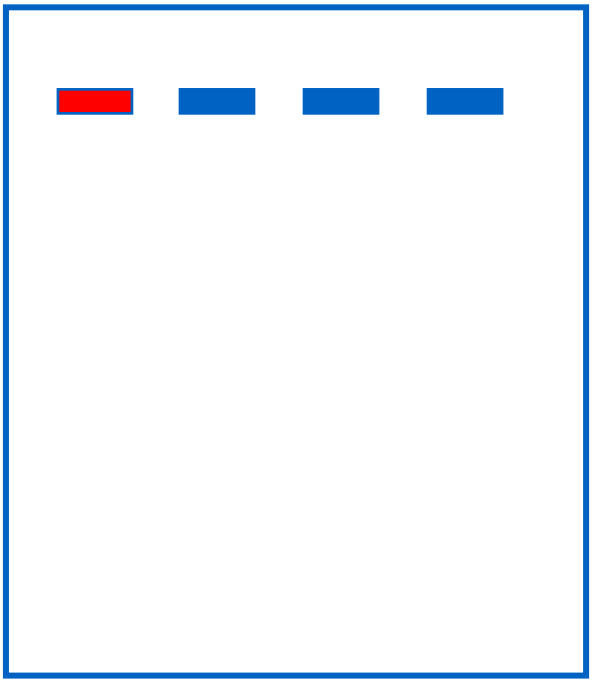
Part B: Gel Electrophoresis- Flow Chart

Laboratory 1.2, Part B Flowchart



Some tools of the trade: Discussion

Here is a schematic of your agarose gel. Annotate (that means mark) the polarity (charge) and the loading order. Draw in where the bands were when the gel finished.



The dyes you are separating are orange G (yellow), bromophenol blue (purple) and xylene cyanol (blue).

1. Based on the results of the gel, which solution (S1, S2 or S3) contained a single dye?

2. Based on the results of the gel, what charge are dyes in solutions S1, S2 and S3?

3. If all three dyes have a similar shape and charge which dye has the smallest molecular weight?

Go onto the internet and search for an image of the three dyes on an agarose gel. Does your image agree with the internet image?

HOW DO YOU BEGIN TO CLONE A GENE: INTRODUCTION

In the Program Introduction, you learned that the increase in diabetes has resulted in a great demand for its treatment, insulin. You also learned that the best way to meet this demand is to insert the human insulin gene into bacteria, enabling the bacteria to produce the insulin protein in quantities large enough to meet the demand. Chapter 1 gave you a chance to work with two physical tools and techniques of genetic engineering that are used to clone a gene: the micropipette and gel electrophoresis. In this chapter you will work with two other important genetic engineering tools—*plasmids* and *restriction enzymes*. These "tools" are biomolecules found in many bacteria, and their discovery was crucial to genetic engineering. With these tools, scientists can modify organisms to make human insulin and other medicines. You will now learn more about these tools and carry out the first steps in your quest to clone a gene.

Learning Goals

- Describe the characteristics of plasmids.
- Explain how plasmids are used in cloning a gene.
- Describe the function of restriction enzymes.
- Explain how to use restriction enzymes to create a recombinant plasmid.

What do you already know?

1. The structure of DNA. Consider the following questions:
   - What is the backbone composed of?

   - Where is the information stored? How is this information protected from the cell's chemicals?

2. Most living things use DNA as their store of genetic information. In what ways is DNA from different organisms the same, and in what ways does it vary?

3. Using your understanding of genes and how they are expressed, explain why it is possible for a bacterial cell to make a human protein from the instructions encoded in a human gene.

Plasmids and restriction enzymes

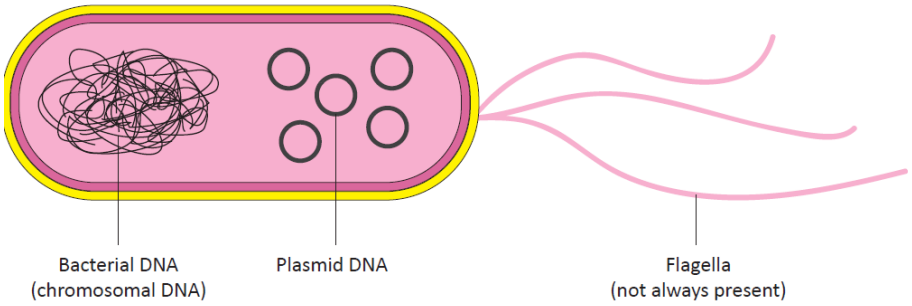
The discovery of plasmids and restriction enzymes in bacteria is a classic example of how findings from basic research can revolutionise a field. Without the discovery of these biomolecules, major

breakthroughs in understanding fundamental processes of life and in developing life-saving products might never have occurred.

Plasmids

Many different types of bacteria carry two forms of DNA: (1) a single chromosome made up of a large DNA molecule that contains all the information needed by the organism to survive and reproduce, and (2) plasmids, which are small circular DNA molecules, ranging in size from 1,000 to 200,000 *base pairs*—two nitrogenous bases joined to connect complementary strands of DNA—that are present in multiple copies separate from the chromosomal DNA (see **Figure 2A.1**). Some bacteria carry as many as 500 plasmids in each cell.

Figure 2A.1: DNA in bacterial cells



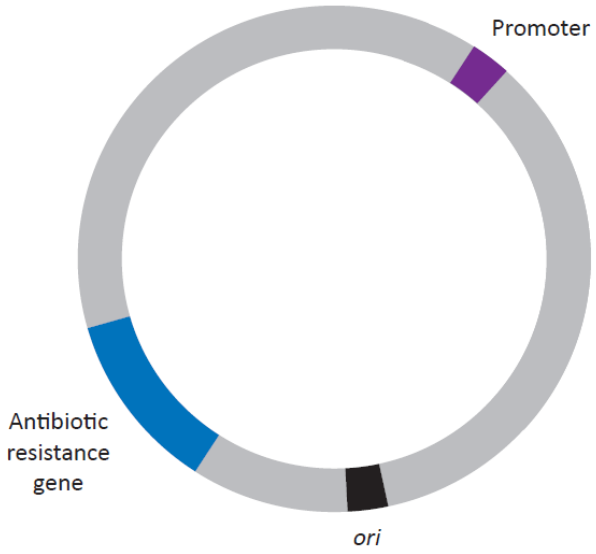
Several characteristics of plasmids make them ideal *vectors* (vehicles for carrying DNA sequences from one organism to another) for genetic engineering, for example:

The ability to *replicate*, that is, to make copies of itself independently of the bacterial chromosome. To do this, plasmids have a specific sequence where the host cell DNA synthesis enzymes bind and initiate *DNA replication* (a biological process that occurs in all living organisms to make copies of their DNA). This sequence is called the *ori* ("*origin of replication*") site.

The ability to initiate *transcription* (the process by which information encoded in DNA is transferred to *messenger RNA* using the host cell *RNA polymerase*). This ability requires another specific sequence, called the *promoter* sequence. The promoter sequence binds RNA polymerase; this is where transcription is initiated. All genes have promoter sequences located next to them in the DNA. In order for genes such as the insulin gene to be expressed in bacteria, they must be inserted in the plasmid next to the promoter sequence.

A gene or genes that code for **resistance to *antibiotics***, a class of compounds that kill or inhibit the growth of microorganisms. These genes code for proteins that inhibit the action of antibiotics secreted by microorganisms and can confer a selective advantage in nature to plasmid-containing bacteria in a microbial population in which bacteria compete for survival.
Figure 2A.2 illustrates some of the characteristics of plasmids that make them ideal vectors for genetic engineering.

Figure 2A.2: A plasmid vector



The basic components of a plasmid are the *ori* site for initiation of DNA replication, a promoter for the initiation of transcription, and a gene for *antibiotic resistance* (the state in which bacteria are no longer sensitive to an antibiotic and will continue to grow and divide in the presence of the antibiotic).

The plasmid you will work with in this and subsequent labs contains the gene for resistance to the antibiotic ampicillin. This gene produces proteins that inactivate the target antibiotic by chemically modifying its structure.

A fourth feature of plasmids that is critical for genetic engineering is that they can be **taken up** by bacteria. When a plasmid with a gene for antibiotic resistance is taken in by bacteria lacking that plasmid, the bacteria will then become resistant to that specific antibiotic. Transformation occurs with a very low efficiency; that is, only a small percentage of bacteria in a population can take in plasmid DNA at any point in time. The presence of an antibiotic resistance gene on the plasmid vector allows us to identify the small percentage of bacteria that took in the plasmid. Bacteria that did not take in the plasmid will be killed by the antibiotic. Those that have the plasmid with the gene of interest will survive and grow.

Once scientists recognised the power of plasmids as a potential vector, the next challenge was to determine how to incorporate a foreign gene of interest, such as the insulin gene, into the plasmid DNA.

Plasmids - Questions

1. What are plasmids?

2. What are the features of an ideal plasmid used for cloning? Why would these features be included in cloning plasmids?

3. How will ampicillin be used in subsequent experiments?

4. How are plasmids used in genetic engineering?

Restriction enzymes

In the early 1950s, scientists observed that certain strains of *E. coli*, a common bacterium found in the human gut, were resistant to infection by *bacteriophage* (viruses that infect bacteria by injecting their DNA into the cell and commandeering the host cell's molecular processes to make more bacteriophage). Investigation of this primitive "immune system" led to the discovery of restriction enzymes, proteins that restricted the growth of bacteriophage by recognising and destroying the phage DNA without damaging the host (bacterial) DNA. Subsequent studies demonstrated that restriction enzymes from different strains of bacteria cut DNA at specific sequences. These sequences are called *recognition sites*.

Table 2A.1 provides examples of restriction enzymes isolated from different strains of bacteria and the DNA sequences they cut. In the examples shown, the enzymes cut asymmetrically on the strands of DNA, leaving single-stranded overhanging sequences at the site of the cut. For example, a cut (or *digestion*) with *Eco*RI will leave an AATT overhang (or "*sticky end*") on one strand and a TTAA sticky end on the other strand.

Table 2A.1: Restriction enzymes used in this laboratory

Source	Restriction enzyme	Recognition site
Escherichia coli	EcoRI	5′ G↓AATTC 3′ 3′ CTTAA↑G 5′
Bacillus amyloliquefaciens	BamHI	5′ G↓GATCC 3′ 3′ CCTAG↑G 5′
Haemophilus influenzae	HindIII	5′ A↓AGCTT 3′ 3′ TTCGA↑A 5′

The symbols ↑ and ↓ indicate where the DNA is cut.

Restriction enzymes: Questions

1. What are restriction enzymes?

2. What is a recognition site?

3. What is the difference between sticky ends and blunt ends? How is this achieved?

4. How do bacteria protect their own DNA when “restricting” foreign DNA?

Cloning: Questions

1. What is cloning?

2. When selecting which restriction enzyme(s) to use what must you consider?

CLONE THAT GENE: Dry Lab

You now know about two biological tools for cloning a gene:

- ✓ A plasmid that has several important features: A restriction enzyme site or sites that opens the plasmid circle and enables insertion of the gene of interest into the plasmid DNA.
- ✓ A sequence for the initiation of DNA replication, called the *ori* site, that allows the plasmid to replicate in the bacteria using the host DNA synthesis enzymes.
- ✓ A promoter sequence for initiating transcription of the inserted gene.
- ✓ A gene encoding a protein for antibiotic resistance, which allows for identification of bacteria that have taken in the plasmid.
- ✓ Restriction enzymes for the digestion of both the plasmid and the human DNA containing the gene of interest (such as insulin) to be cloned

How do scientists use these two tools to create a recombinant plasmid, which contains the insulin gene (or any other gene of interest) inserted into a bacterial plasmid? One important step is choosing a restriction enzyme or enzymes that cut the plasmid and the human DNA. The restriction enzyme(s) must do all of the following:

- ✓ Cut the plasmid at a site or sites that allow for the insertion of the new gene.
- ✓ Cut the plasmid at an appropriate site to ensure that no important genes or sequences are disrupted, including the *ori* site, the promoter and at least one of the genes encoding antibiotic resistance.
- ✓ Cut the plasmid near the promoter so that the inserted gene can be expressed.
- ✓ Cut the human DNA as close as possible to both ends of the gene of interest so it can be inserted into the appropriate site in the plasmid DNA, without cutting within the gene.
- ✓ Obtain handouts, scissors and sticky tape to complete the lab.

Procedure

1. On the **Plasmid Diagram**: Use scissors to cut out the plasmid sequence and tape the ends together to make a paper model of the plasmid.
2. Locate the positions of the *ori* site, the promoter site, and the genes for antibiotic resistance.
3. Locate the positions of each restriction enzyme recognition site.
4. Choose the restriction enzyme that should be used to cut the plasmid.
5. Verify that the restriction enzyme meets all the following criteria:
   - ✓ The *ori* site on the plasmid is intact.
   - ✓ The promoter site is intact.
   - ✓ At least one of the antibiotic resistance genes is intact.
   - ✓ The enzyme cuts the plasmid only once.
   - ✓ The cut is close to the promoter sequence.
6. Review **Table on page 23** and use scissors to cut the plasmid at the recognition site exactly as the restriction enzyme would cut it. Write the sequences of the nucleotides that are left on each end of the plasmid.

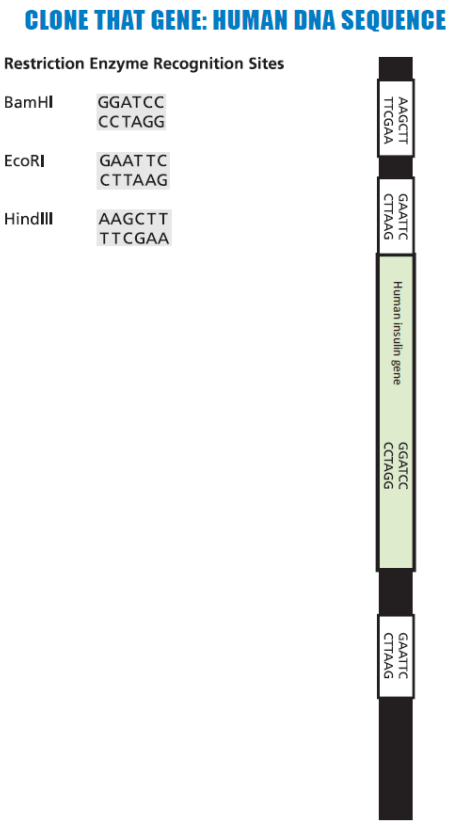
7. On the **Human DNA Sequence** scan the human DNA sequence and determine where the three restriction enzymes, *Bam*HI, *Eco*RI and *Hind*III, would cut the DNA.

8. Determine whether the restriction enzyme you chose in step 2 is a good choice for cutting out the insulin gene from the human DNA by verifying that it meets all the following criteria: It does not cut within the insulin gene.

   - ✓ It cuts very close to the beginning and end of the gene.
   - ✓ It will allow the insulin gene to be inserted into the cut plasmid.

9. Review **Table on page 25** and use scissors to cut the human DNA at the recognition site exactly as the restriction enzyme would cut it. Write the sequences of the nucleotides that are left on each end of the insulin gene after it is cut from the human DNA.

10. Use tape to insert the insulin gene into the cut plasmid. Verify that the sticky ends will connect in the correct orientation (in the lab, a third biological tool, *DNA ligase*, is used to permanently connect the sticky ends together). This is a paper model of a recombinant plasmid that contains an insulin gene. Once the plasmid replicates (copies) itself, the insulin gene is also copied, or cloned!



Dry Lab: Discussion Questions

1. What are the advantages of using two restriction enzymes?

2. What does the ampicillin resistance gene code for? Why is it included in the plasmid?

3. What is the promoter region? What does it do?

4. What is *araC* and why might this be included in the plasmid?

GETTING RECOMBINANT PLASMIDS INTO BACTERIA: INTRODUCTION

A plasmid is an ideal vector for carrying DNA sequences from one organism to another. The plasmid is equipped with (1) a promoter that enables gene transcription, (2) a sequence for the initiation of DNA replication, and (3) an antibiotic resistance gene. The plasmid can be taken up by bacteria where it replicates, and its genes are expressed using the bacterial cellular machinery. If a gene of interest has been inserted into the vector, the bacteria produces the product encoded by that gene.

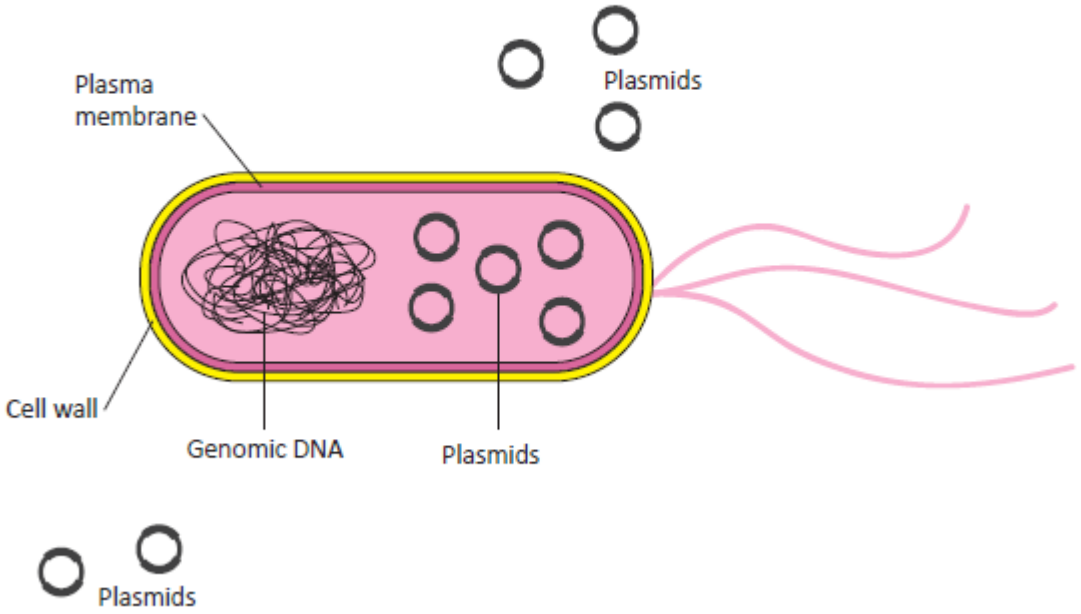
Learning Goals

- Describe the role of transformation in the gene cloning process.
- Explain the purpose of each control in the transformation experiment.
- Explain how the information encoded in a gene is expressed as a *trait.*

Bacteria Transformation

Once a recombinant plasmid is made that contains a gene of interest, such as insulin, the plasmid can enter bacterial cells by a process called transformation. **Figure 5A.1** illustrates transformation.

Figure 5A.1: Bacterial transformation



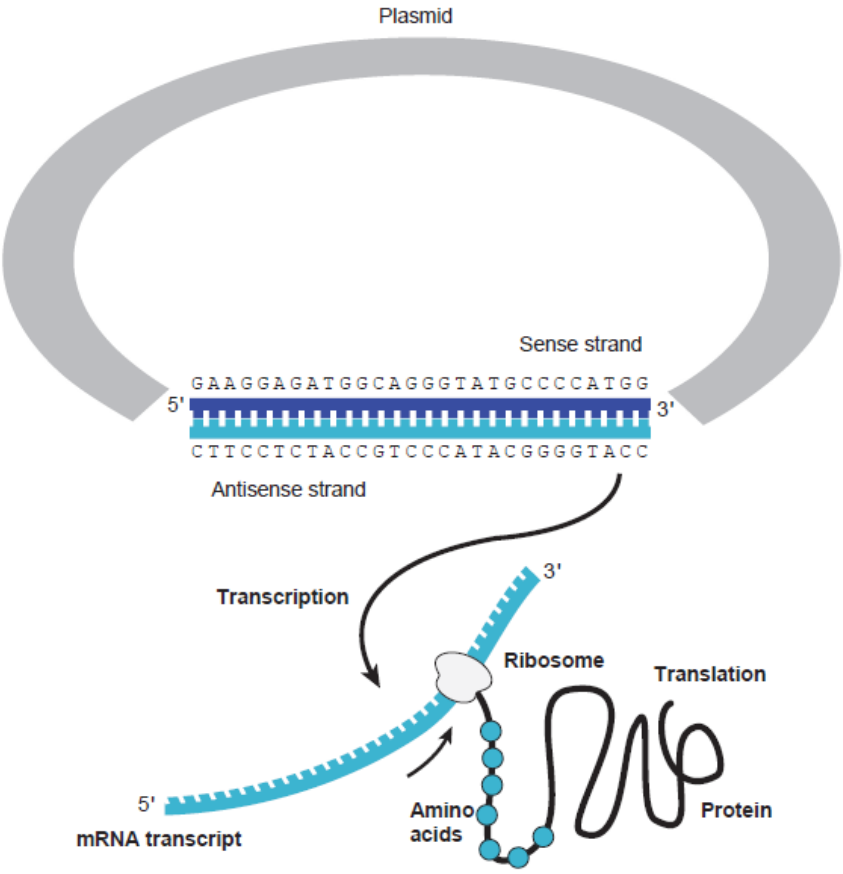
The uptake of DNA from the environment of a bacterial cell occurs with a very low efficiency in nature. *E. coli* bacteria have complex plasma membranes that separate the external environment from the internal environment of the cell and carefully regulate which substances can enter and exit the cell. In addition, the cell wall is negatively charged and repels negatively charged DNA molecules.

From plasmid to DNA protein

Once a recombinant plasmid has entered the bacterial cell, DNA polymerase initiates replication at the *ori* site, and the plasmid replicates using the bacterial DNA replication enzymes. These multiple copies of plasmids can now produce the protein of interest, such as insulin, in quantity. In this

process, the information encoded in the human DNA is transferred from DNA to protein using the transcription and translation machinery of the cell (see **Figure 5A.2**). The protein then alters the observable traits of the organism.

Figure 5A.2: Gene expression from a plasmid in the bacterial cell



Genetic engineering is only possible because genes from different organisms can be expressed in bacteria. On Earth, all life is related, and the way that information is encoded in DNA is universal. As you may already know, proteins are made up of smaller subunits called *amino acids*, and a sequence of three nucleotides in DNA code for a single amino acid. These three-nucleotide sequences are called *codons*. For example, the codon TTG codes for the amino acid tryptophan, whereas the codon AAG codes for the amino acid lysine. In many cases, more than one codon can encode the same amino acid. For example, AAA is also a codon for lysine. In addition, there are informational codons, such as the *start codon* (ATG) and the *stop codon* (TTA), which show where in the DNA sequence the code for the protein begins and ends.

So far in your quest to clone a gene you have verified that you have the pARA-R plasmid containing the *rfp* gene that can make the *red fluorescent protein*. In this laboratory you will carry out another step of the gene cloning process, which is to transform *E. coli* bacteria with this plasmid. Using *E. coli* bacteria that have been pretreated with calcium chloride, you will divide the bacteria into two groups: a control group to which no plasmid is added, and a treatment group to which you add the

pARA-R plasmid. After heat-shocking both groups of cells, you will grow them under several different conditions:

1. In the presence of Luria Broth (a *medium* that supports bacterial growth).
2. In the presence of Luria Broth and the antibiotic ampicillin.
3. In the presence of Luria Broth, ampicillin and the sugar arabinose.

By examining the growth of bacteria under these conditions, you can verify that your procedure worked, and you can identify the bacteria transformed with the pARA-R plasmid. How will you know if you are successful? The bacteria will have a new and highly visible trait: It will now produce red fluorescent protein, which makes the cells red or bright pink!

1. What are the steps when a cell transcribes and translates a gene (DNA) into the polypeptide (protein) it encodes? *This is essential for answering HSC exam questions.*

2. What is the relationship between genes (the genotype), proteins and traits (the phenotype)?

3. What do humans and bacteria (and for that matter all living organisms as well as a few non-living viruses) have in common that enables cloning?

Terminology and abbreviations used in this lab session:

1. The CC tube contains 100 µL of competent cells. What are these?

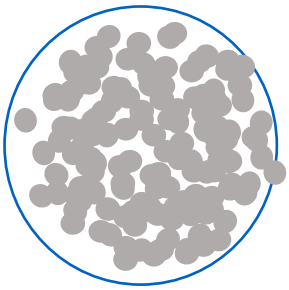
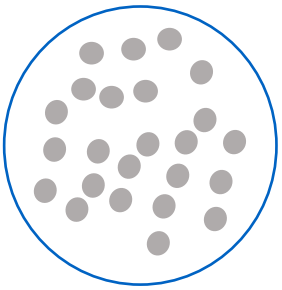
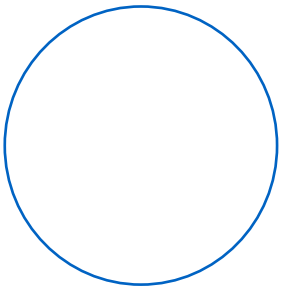
2. What is the RP tube?

3. What is P+ and P-?

4. You will be given three plates: LB, LB/amp and LB/amp/ara. What are the differences between the three plates and what information will each component tell us?

Read over the lab protocol before you start the lab and make sure you can answer the previous questions.

Once you are confident with the terms and abbreviations, predict the bacterial growth on each of the plates. High growth will have lots of colonies, medium growth scattered colonies and no growth will be a blank plate.

High growth Lots of colonies, often overlapping (+++)	**Medium or low growth** Scattered colonies, usually discrete. Medium (++) or low (+)	**No growth** Blank plate (-)
		

SAFETY

- All appropriate safety precautions and attire required for a science laboratory should be used. Please refer to your teacher's instructions.
- Use caution when handling *E. coli* bacteria and use aseptic technique.
- Aseptic technique is a set of procedures that ensure protection of the lab worker and protection of a bacterial sample, which is necessary for the experiment to be successful.

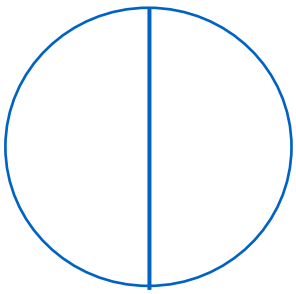
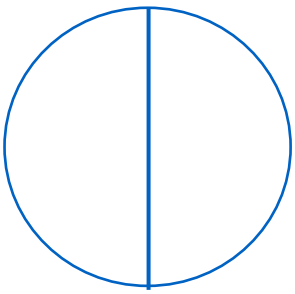
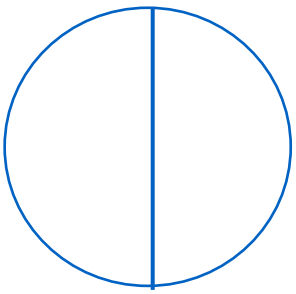
Specifically …

- Do not touch anything that has been or will be in contact with *E. coli* bacteria.
- Students handling equipment that contacts bacteria should wear gloves.
- Try to avoid spills or contamination of surfaces with anything that has been in contact with *E. coli* bacteria. Immediately inform your teacher if a spill or contamination occurs.
- When you have finished using microfuge tubes, pipette tips, and cell spreaders, place them immediately into the biohazard bag or waste container, as directed by your teacher.
- When directed to do so, place your Petri plates in the biohazard bag.
- Wash your hands well with soap after completing the lab.

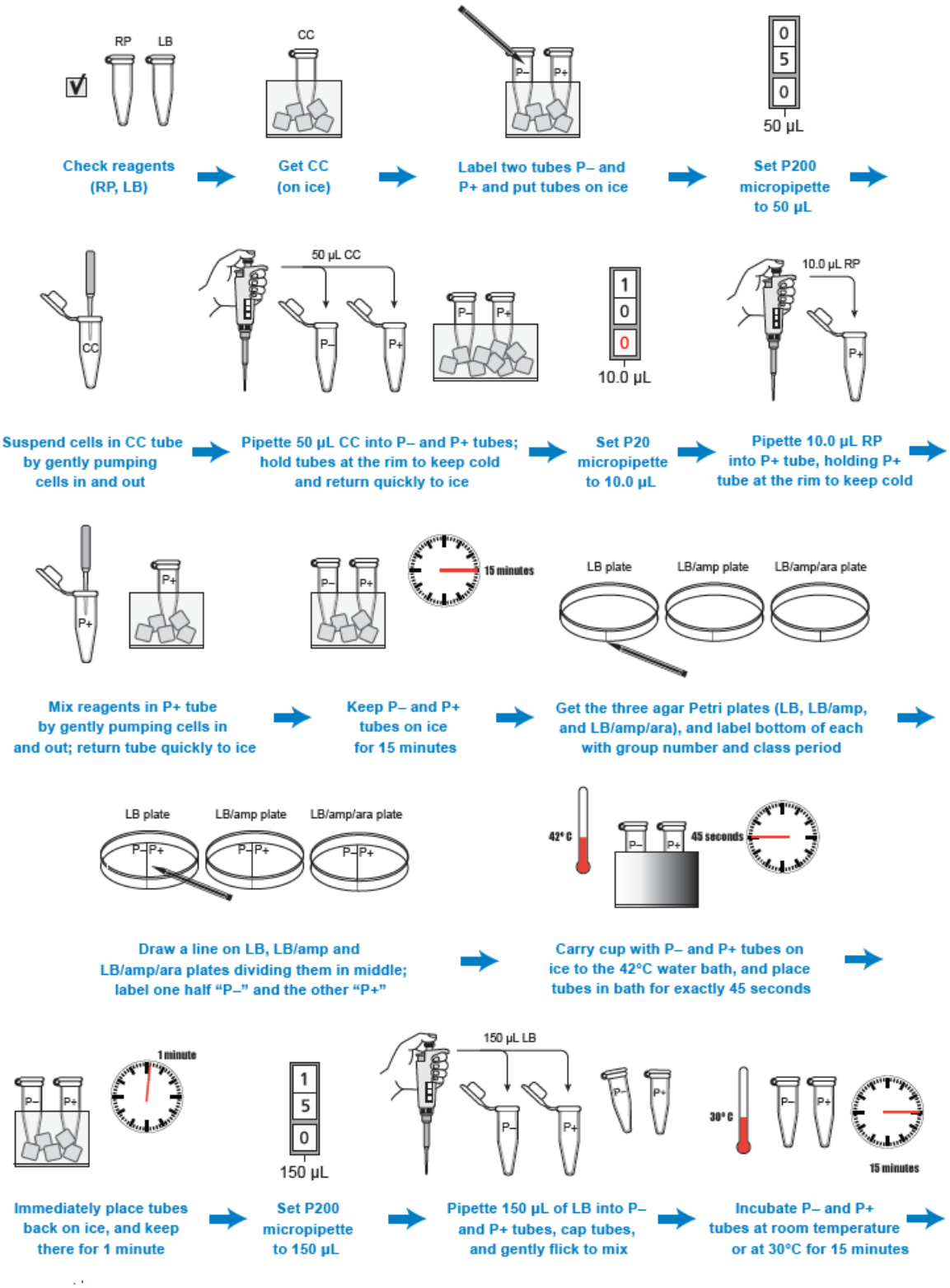
Laboratory Techniques

- The CC tube must always be kept on ice.
- Fill a cup with some of the crushed ice from the container holding the CC tubes before taking a CC tube. You'll need to keep the CC tube always on ice.
- The competent cells in this lab must be kept cold—be sure to pick up microfuge tubes by the upper rim to avoid warming the cells.
- Bacterial transformation requires sterile techniques. It is essential that these directions be followed precisely.
- To avoid contamination, be sure to use a new micropipette tip for each addition.

Getting Recombinant Plasmids into Bacteria: Predictions

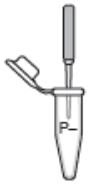
LB PLATE	LB/AMP PLATE	LB/AMP/ARA PLATE
P- | P+ 	P- | P+ 	P- | P+ 
Explain your prediction for each plate:		
P-	**P-**	**P-**
P+	**P+**	**P+**

Getting Recombinant Plasmids into Bacteria: Flow Chart

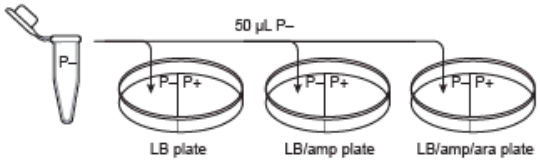


Getting Recombinant Plasmids into Bacteria: Flow Chart (continued)

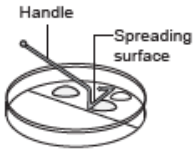
Set P200 micropipette to 50 µL



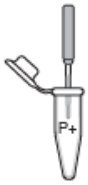
Gently pump pipette a couple of times in P– tube



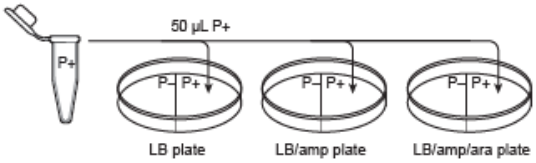
Pipette 50 µL of P– onto P– halves of LB, LB/amp and LB/amp/ara plates and close lids immediately after dispensing P–



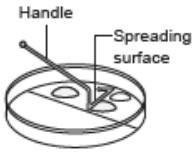
Use one cell spreader to spread P– cells on P– halves of plates in this order: LB (1st), LB/amp (2nd), LB/amp/ara (3rd), and close lids



With new pipette tip, gently pump pipette a couple of times in P+ tube



Pipette 50 µL of P+ onto P+ halves of LB, LB/amp and LB/amp/ara plates, and close lid after dispensing P+



Use one cell spreader to spread P+ cells on P+ halves of plates in this order: LB (1st), LB/amp (2nd) and LB/amp/ara (3rd), and close lids

Let plates sit right side up for 5 minutes

Parafilm and tape all three plates together, and label tape with group number and class period

Place plates in the 30°C incubator upside down

Incubate plates for 24–36 hours at 30°C

Examine plates and record the amount of growth on each

Getting Recombinant Plasmids into Bacteria: Questions

1. Draw or insert an image of your plates after incubation below. Did the actual results agree with your predictions? If they didn't, can you explain what might have happened? What would you change next time you did the experiment?

2. Did you get red colonies on any of the plates? If so which plate?

3. If the competent cells did not take up the plasmid what would the plates look like?

4. If you accidently contaminated the P- tube with plasmid what would the results look like?

5. You have a few colonies growing on the LB/amp P- side of the plate (nowhere near as many as the P+ side). How might you explain this observation?

6. Why would the same bacterial culture appear red on one plate and not on another?

7. Would the red fluorescent protein (RFP) produced by the sea anemone (expressed from the *rfp* gene contained in the sea anemone's genome) have the same amino acid sequence as the RFP produced by the recombinant bacteria containing the *rfp* gene? How is this possible?

Using PCR to amplify the *rfp* gene: Introduction

The most widely used method in biotechnology is the polymerase chain reaction, called PCR. PCR provides a way to identify a specific DNA sequence quickly and accurately and then make multiple copies of that sequence. PCR uses an enzyme—DNA polymerase—which in cells replicates, or copies, DNA. This method has had a profound impact on biotechnology and has been applied to such areas as genetic engineering, prenatal diagnosis, forensics, medicine, cancer detection, diagnosis of infectious disease and basic research.

In this chapter, you'll learn about the multiple uses of PCR and then carry out the PCR method in the laboratory. The PCR you will carry out is a colony PCR—a quick procedure to identify which, if any, transformed bacterial colonies carry the gene of interest, *rfp*. This work will give you knowledge of and skill in the important method of PCR.

Learning Goals

- Carry out PCR.
- Describe applications of PCR.
- Explain the role of DNA polymerase and DNA primers in PCR.

What is PCR?

Using an enzymatic reaction, PCR allows for the amplification of a specific region of DNA by successive rounds of gene replication. This results in the rapid synthesis of billions of copies of the specific region of DNA. Prior to the development of PCR, the only way to make multiple copies of a specific sequence of DNA was through biological amplification in bacteria, a technique that is materials-, labour- and time-intensive due to the complex extraction techniques needed to purify the DNA. In contrast, DNA amplified by PCR is easy to purify, and the technique requires significantly less labour and fewer materials than biological amplification in bacteria.

The table on the next page shows some of the many uses of PCR. For example, in determining the treatment for acute myeloid leukemia, PCR is used to identify the nature of the cancer-causing mutation. Knowing the specific kind of mutation enables the disease to be treated with a specific drug that results in a good prognosis in patients with this mutation. PCR can also be used in prenatal diagnoses for chromosomal abnormalities, such as trisomy 21, which results in Down syndrome. PCR provides a non-invasive diagnostic by analysing foetal cells in the mother's blood. The results are obtained far more quickly than with karyotype analysis, which requires culturing foetal cells obtained from the amniotic fluid that surrounds a foetus.

MEDICAL	EXAMPLE
Genetic testing (pre- and post-natal)	• Mutations leading to genetic disease (such as sickle cell anaemia, cystic fibrosis, Huntington's chorea and Tay-Sachs) • Chromosomal aberrations (such as duplications or deletions)
Tissue typing	• Prior to organ transplantation to avoid immune rejection
Cancer detection and therapy	• Diagnosing cancers (such as breast and pancreatic) • Determining the origins of cancer during metastasis • Predicting response or resistance to therapy
Detection and identification of pathogenic organisms	• Diagnostic for viruses (such as HIV, HPV and Ebola), bacteria (such as those causing tuberculosis or strep throat) and parasites (such as those causing malaria or trichinosis) • Determining drug sensitivities of infectious agents • Epidemiological studies mapping the spread of infectious diseases
FORENSICS	**EXAMPLE**
Identification of bodies	• Victims of crimes and natural disasters (such as earthquakes and tornadoes)
Identification of suspects of crime	• DNA from blood, semen, skin and cigarette butts or other evidence left at crime scenes
Family relationship testing	• Identifying family relationships, such as the father of a child
Origins	• Determining family lineages e.g. descent from Genghis Khan
BASIC RESEARCH	**EXAMPLE**
Drug discovery	• Examining the effect of a trial drug by measuring the impact on target gene expression and production of enzymes in the body that facilitate distribution or disposal of the drug
Genetic engineering	• Creating transgenic organisms
Molecular anthropology, archaeology and evolution	• Investigating evolutionary links between ancient and modern humans • Bringing back extinct species, for example, the woolly mammoth • Identifying common ancestry among organisms
Patterns of gene expression	• Investigating mechanisms and regulation of embryogenesis • Cell differentiation • Initiation of cancers • Molecular responses to environmental factors • Embryonic and induced pluripotent stem cells (which can differentiate into many cell types)
Genetic mapping	• Determining the physical position of genes within chromosomes; the genetic map produced by the Human Genome Project has helped medical researchers connect genetic diseases with specific gene mutations

Like other biotechnology methods, PCR is based on basic science discoveries. These discoveries, which are related to DNA replication, are as follows:

1. DNA becomes single-stranded at high temperatures because its two strands separate (denature).
2. Short DNA single-stranded primers (15 to 30 nucleotides in length) can stick, or anneal, to specific sequences in single-stranded DNA and provide the 3′end of DNA required for replication. Annealing can occur only when the strands have complementary base pairs.
3. One specific kind of DNA polymerase, Taq DNA polymerase, can function at temperatures higher than most polymerases (this enzyme was isolated from thermophilic bacteria).

The discovery of the Taq DNA polymerase and its ability to operate at high temperatures was a vital step in developing PCR because high temperatures are required to denature the DNA and make the nucleotides accessible for annealing. The denaturation step provides an opportunity—the physical space—for primers to bind. The annealing temperature ensures that only primers with the exact sequences will anneal and allow replication at the desired site.

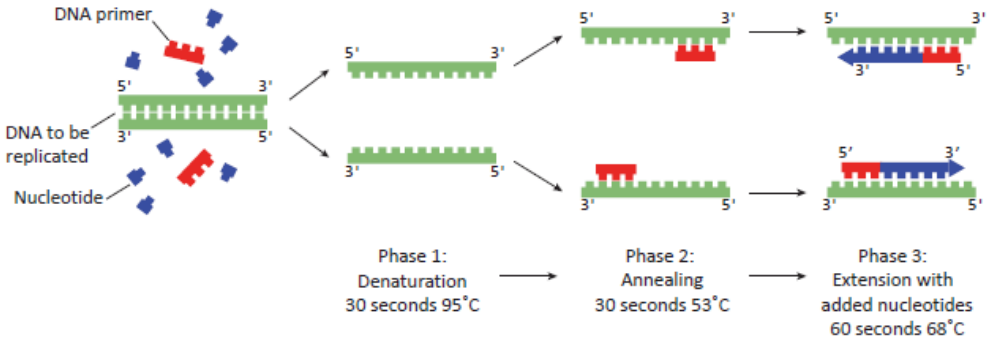
In this laboratory, you will use PCR and gel electrophoresis to examine the DNA from the colonies produced and confirm that the cells producing red fluorescent protein have been transformed with the plasmid carrying the *rfp* gene, pARA-R. The sizes of the DNA fragments can be determined by comparing them to a DNA ladder—a mixture of DNA fragments with known sizes (when the DNA ladder is run on gel electrophoresis and stained, the bands that show the fragments look like the rungs of a ladder). The DNA ladder is loaded adjacent to other DNA samples, which makes it easy to compare the bands in the samples with the bands in the ladder. The results from the gel electrophoresis will provide evidence that the transformed bacterial colonies carry the gene of interest.

PCR Steps

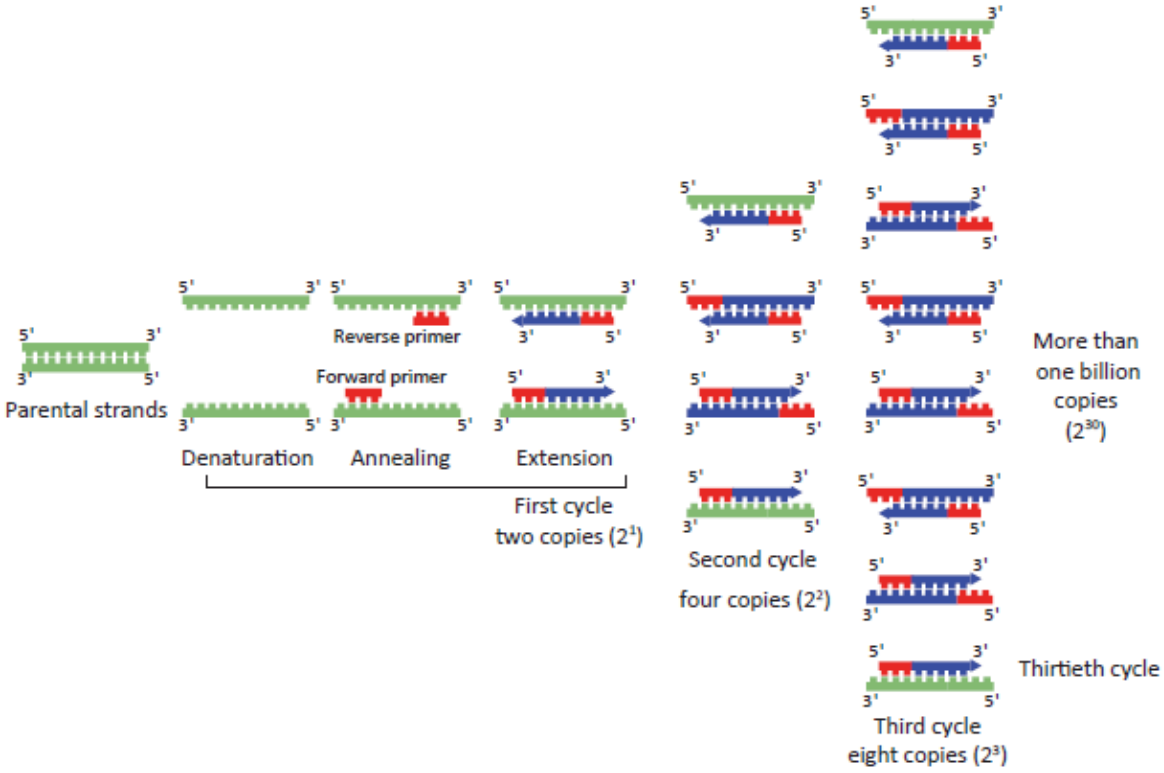
PCR is an important method used to amplify DNA by multiple rounds of replication. The steps in carrying out PCR are as follows (see Figure E.1):

1. *Denaturation phase*: The DNA sample containing the sequences to be amplified is denatured at 95°C, making it single-stranded.
2. *Annealing phase*: Single-stranded DNA primers are annealed to the denatured single-stranded DNA at 53°C.
3. *Extension phase*: Taq polymerase replicates the region of interest at 68°C, the optimal temperature for this enzyme, by adding nucleotides to the 3’end of the primers.

Figure E.1: Three Steps of the PCR

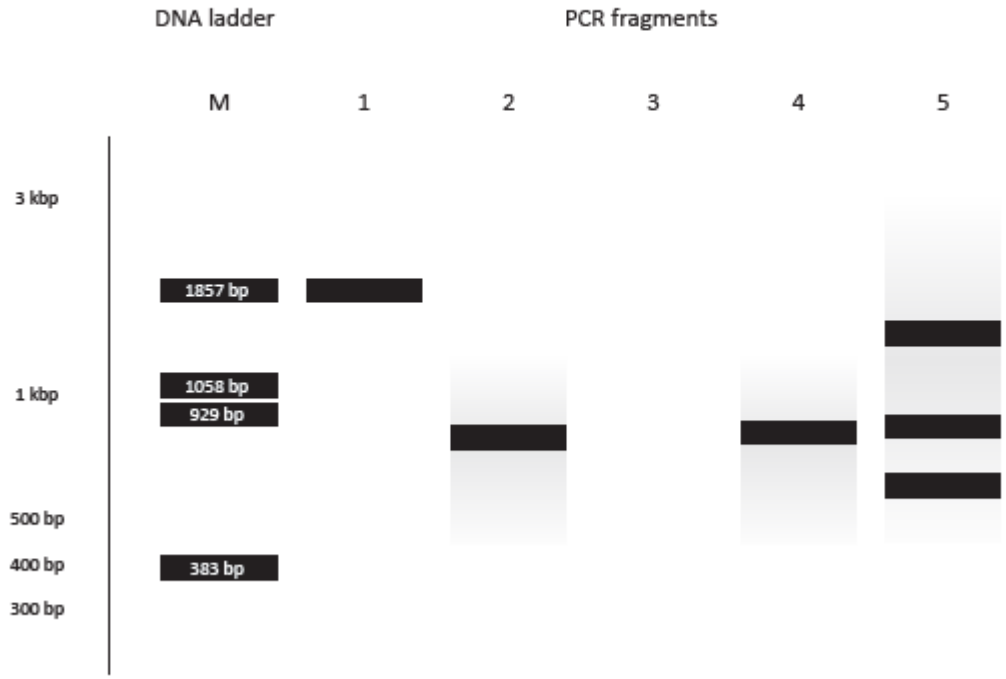


PCR is carried out in an instrument called a thermocycler, which controls the temperature at each step of the reaction. The three steps constitute one synthesis cycle, which takes about three minutes, during which the number of copies of the region of interest doubles. A reaction that runs for 30 cycles can result in more than one billion copies.



The success of the reaction is then determined by using gel electrophoresis to analyse the products (see **Figure E.3**). The purpose of running an analytic gel is to ensure that a product has been made, the product is the expected length, and only one product band has been synthesised.

Figure E.3: Verification of PCR Product



Using PCR to amplify the *rfp* gene: Introduction questions

1. When might it be important to copy DNA quickly? *There some very recent topical examples.*

2. How does PCR work?

Colony PCR

We will be performing Colony PCR to confirm that our plasmid contains the *rfp* gene of interest. This is a much quicker way of gene confirmation than the plasmid isolation and restriction digestion performed. Your starting material will be white cells and red cells taken from LB/amp/ara plates. Both colonies will contain cells with a plasmid.

Using the plasmid diagram below to record the sizes of the amplified product (amplicon) expected using both pARA and pARA-R as templates.

Plasmid	Amplicon (bp)
pARA	
pARA-R	

Sizes of the sequences Amplified by PCR

This laboratory uses PCR to amplify sections of the pARA and pARA-R plasmids. Only the sequence that is amplified from pARA-R plasmids contains the *rfp* gene. The sizes of the amplified sequences in each plasmid vary and therefore can be used to identify the two plasmids. The following figures show the important sequences, the position of the primer binding sites and the size of the amplified fragments.

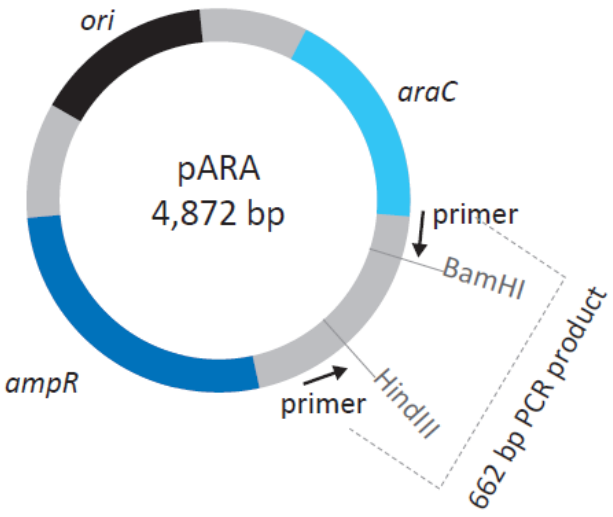
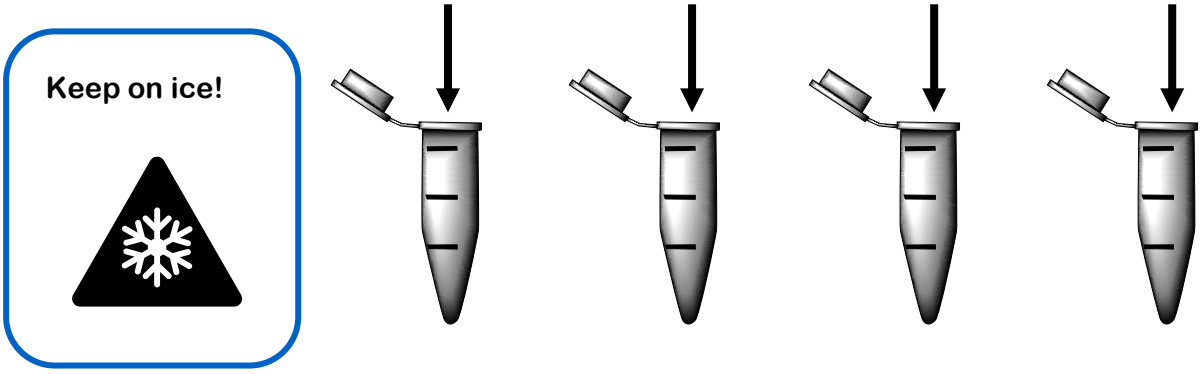


Figure 1: Features of the pARA plasmid, including the *Bam*HI and *Hin*dIII restriction enzyme sites, the forward and reverse primer binding sites, the beta-lactamase gene encoding the protein for ampicillin resistance, and *araC* gene encoding the AraC protein that inhibits the expression of the *rfp* gene in the absence of arabinose. The amplified sequence is 662 bp in size.

Verification Gel Electrophoresis: Flowchart

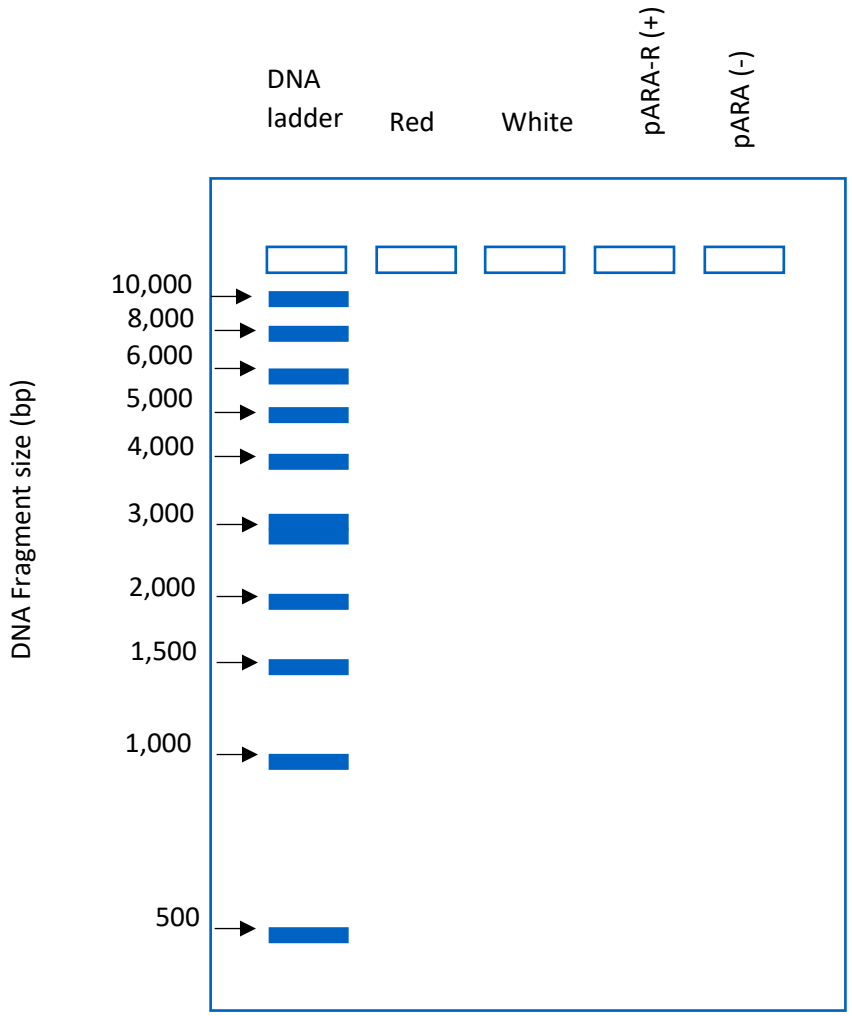
Component		Tube 1 Red colony	Tube 2 White colony	Tube 3 pARA-R +	Tube 4 pARA -
1	Master mix (µL)	23	23	23	23
2	Red colony	*			
3	White colony		*		
4	pARA-R (+) (µL)			2	
5	pARA (-) (µL)				2



- *Pick out one colony with a sterile tip. Make sure the colony is a single discrete colony not touching any other colonies and try not to pick up any agar when you pick the colony. We will assume the colony is ~2 µL.
- Use fresh sterile tips for each of the additions.
- Tube 3 pARA-R is your positive control and Tube 4, pARA is your negative control. *Can you think of any other controls?*
- Once everything is added, label the sides of the PCR tubes and place them in the thermocycler. Make sure you know which tubes are yours in the heating block… a photo often helps here!

Verification Gel Electrophoresis: Prediction

Predict the products from your PCR amplification on the gel schematic below. To do this you will need to refer the table on **page 43**.



Verification Gel Electrophoresis: Method

MATERIALS

Reagents

- 4 PCR tubes with amplification products (from Part A) in rack
- Microfuge tube of loading dye (marked “LD”)
- Microfuge tube of DNA ladder (marked “M”)
- Plastic microfuge tube rack
- P20 micropipette
- Green box of white disposable pipette tips
- Electrophoresis box loaded with 0.8% agarose gel (will be shared among groups)
- 500-mL flask containing 300 mL of 1x SB buffer (will be shared among groups)
- Waste container for used tips and microfuge tubes (will be shared among groups)
- DNA Ladder Diagram (Worksheet E) (one for each group member)

SAFETY:

- *Use all appropriate safety precautions and attire required for a science laboratory, including safety goggles. Please refer to your teacher's instructions.*
- *Wash your hands well with soap after completing the lab.*

METHODS

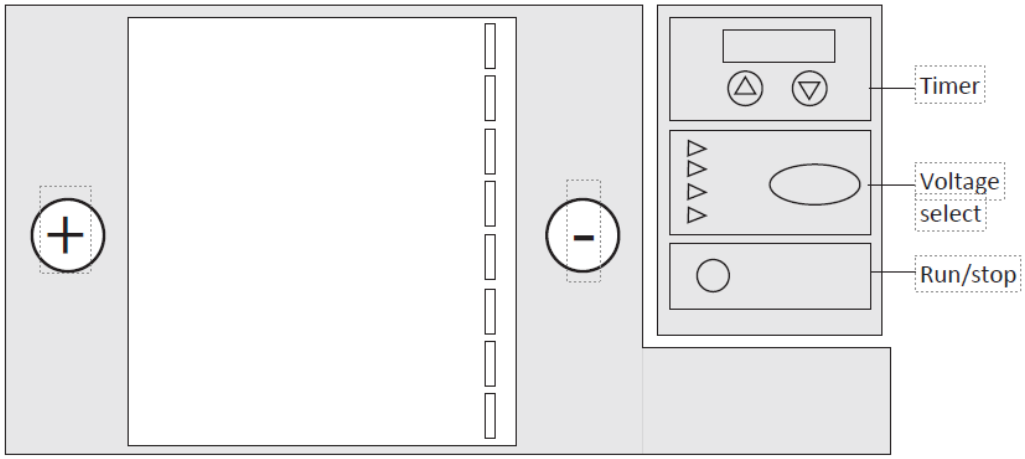
1. Obtain your PCR tubes from Part A and check your rack to make sure that you have the reagents listed.
2. Add 2 µL of loading dye ("LD") to each of the four PCR tubes. Gently pump each tube to mix the loading dye with the DNA without creating bubbles.

NOTE*: Be sure to use a new micropipette tip for each reagent to avoid contamination.*

3. Make sure that the wells in your gel electrophoresis unit are located near the negative electrode.
4. Fill the box with 1x SB to a level that just covers the entire surface of the gel.
5. Make a drawing in your notebook that shows the location of the wells in the electrophoresis box. The order of the samples in each well is as follows:
    a. DNA ladder (M)
    b. Red colony (1)
    c. White colony (2)
    d. pARA-R (3)
    e. pARA (4)
6. Using a fresh pipette tip for each sample, dispense **10.0 µL** of each sample and of the DNA ladder into their designated wells. For each sample, do the following:
    a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
    b. Lower the pipette tip until it is under the buffer but just above the well.
7. When you have loaded all the samples, close the cover tightly over the electrophoresis box.
8. Plug in the power supply cord on the electrophoresis box. Set the voltage to 100V. Set the timer to **35 minutes**. Press the Run/Stop button once to start the electrophoresis. See Figure E.4.
9. After two or three minutes, check to see if the dyes are moving toward the end
10. Let your gel run until the pink/red dye is approximately halfway down the gel. At this point stop the electrophoresis and unplug the power supply.
11. Carefully remove the cover from the gel box and transfer the gel in the transparent gel tray into the PrepOne™ gel illuminator.

12. Turn on the gel illuminator to view your DNA bands. Use the photo hood and capture an image of your gel.

Figure E.4: Electrophoresis



Verification Gel Electrophoresis: Questions

1. Why is it important to examine the PCR products?

2. Study your gel from Laboratory E. How did your actual gel results compare to your gel predictions?

3. Do you see any bands that were not expected? What could explain the origin of these unexpected bands?

4. Does the gel photograph show that your PCR was successful? Describe the evidence you used to make this assessment.

5. In this laboratory you used two controls. Can you think of any additional controls this laboratory might have included? Explain.

6. Why is denaturing of DNA needed to carry out PCR?

7. What are the roles of DNA polymerase and DNA primers in the PCR method?

Verification Gel Electrophoresis: Your results

Insert your gel image here:

Applications of Biotechnologies Note-making Scaffold

Learning Goals:

- Research three additional examples of biotechnologies that genetically modify organisms

Use the following scaffold to make notes about three different applications of biotechnologies to genetically modify organisms. Some examples of biotechnologies can be found on the biotechnology timelines and in the table of PCR application on pg 40. Some research resources to get you started can be found are in the LabXchange Pathway link below. You can also use other resources of your choosing.

https://www.labxchange.org/library/pathway/lx-pathway:adfaa302-f85d-4e0c-bd69-55e49600e298

Name of the Biotechnology that genetically modifies organisms	
What problem is the biotechnology solving?	
How does the biotechnology work?	
How has an understanding of the structure of DNA lead to the development of this technology?	
What concepts do you need to clarify? **Or** **What Questions came to mind while you were researching this biotechnology?**	

Name of the biotechnology that genetically modifies organisms.	
What problem is the biotechnology solving?	
How does the biotechnology work?	
How has an understanding of the structure of DNA lead to the development of this technology?	
What concepts do you need to clarify? **OR** **What questions came to mind while you were researching this biotechnology?**	
Name of the biotechnology that genetically modifies organisms.	
What problem is the biotechnology solving?	
How does the biotechnology work?	
How has an understanding of the structure of DNA led to the development of this technology?	
What concepts do you need to clarify? **OR** **What questions came to mind while you were researching this biotechnology?**	

Extended Response Scaffold and Marking Guideline

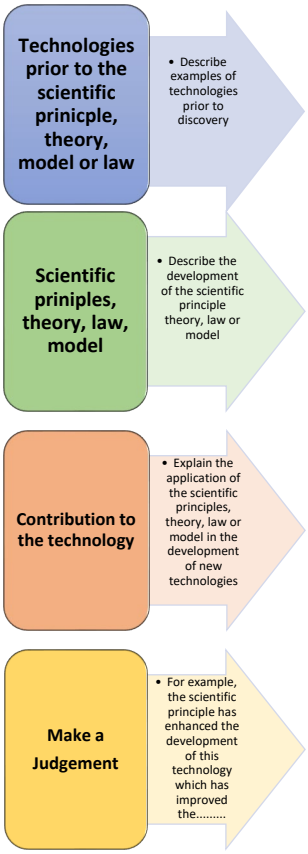
Assess the impact of the discovery and understandings of the structure of DNA on the development of biotechnologies to genetically modify organisms.

Learning Goals:

- Demonstrate understanding of the importance of the discovery of DNA on technologies to genetically modify organisms
- Plan and write an extended response
- Use a marking guideline to self/peer assess and provide warm and cold feedback

Use the following scaffold to help you plan a response the extended response question above. Draw on examples and scientific knowledge gained throughout the AMGEN Biotech Experience. Write your final assessment in a separate document. You are then to self/peer mark your responses with the provided marking scheme and give yourself/peer warm and cold feedback.

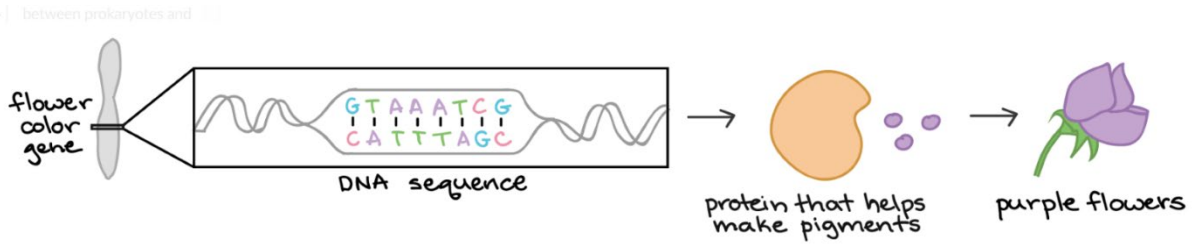
Thinking /processing	**Assessment of impact of scientific theories, laws, and models on of the development of technology**



Marking Scheme: Impact of scientific understanding on technology
7–8 Marks: • Provides detailed characteristics and features of examples of biotechnologies prior to the discovery of the structure of DNA. • Provides detailed characteristics and features of the development of understanding the structure of DNA • Make the relationships evident between the understanding the structure of DNA and the development of two or more biotechnologies to genetically modify organism • Makes a relevant judgement about the impact of the scientific theory, model, and/or law on the development of this technology.
4–6 Marks: • Identifies examples of biotechnologies prior to the discovery of the structure of DNA. • Provides characteristics and features of the development of understanding the structure of DNA • Provides examples of biotechnologies to genetically modify organisms developed using an understanding of the structure of DNA • Makes a judgement about the impact of the scientific theory, model, and/or law on the development of this technology.
2–3 Marks: • Identifies examples of biotechnologies prior to the discovery of the structure of DNA. • Identifies the development of understanding the structure of DNA • Identifies an example of biotechnologies to genetically modify organisms
1 Mark: • Provides some relevant information

Applying your understanding of how DNA works

A. To test your understanding of this process, annotate and explain **where** each step occurs in the cell and **what** is happening in this diagram. Try and explain how DNA codes for traits in living things to another student or family member.



B. Scientist have discovered they can produce red flowers by changing the thymine base to an adenine base in the first codon of the DNA sequence that makes the pigment. Annotate the diagram below to show how this is possible.

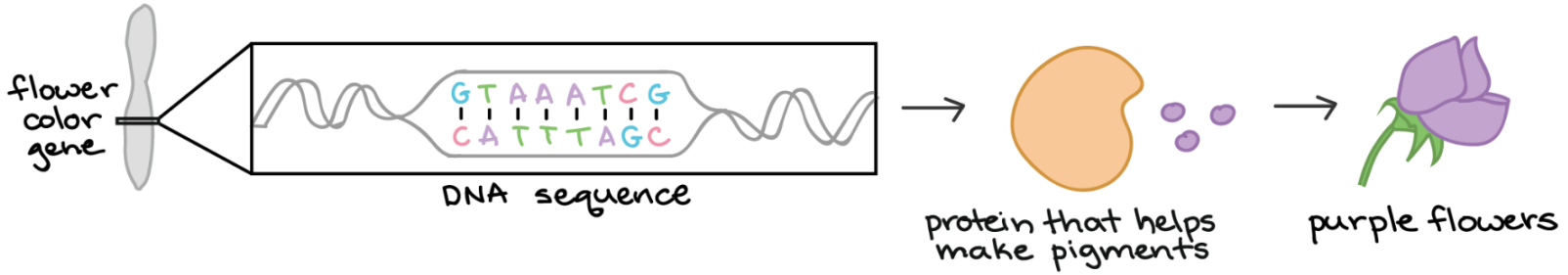


Diagram from Kahn Academy https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/translation/a/intro-to-gene-expression-central-dogma

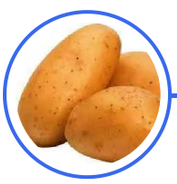
Ancient and Classical Biotechnology Pre-1950

Exploring biotechnology in the realm of history

At its simplest, biotechnology is technology based on biology - biotechnology harnesses cellular processes to develop technologies and products that help improve our lives and the health of our planet. Humans have been manipulating biological processes for thousands of years.
Here is an overview of the evolution of biotechnology:

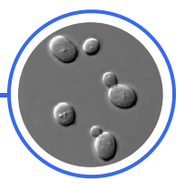
8000 BCE

Humans begin choosing plants and animals so they can be domesticated. Potatoes become the first cultivated food.



7000 BCE

The oldest use of yeast fermentation was used to make beer in Sumeria and Babylonia.



4000 BCE

Egyptians use yeast for bread and wine making.

3000 BCE

The mule is the oldest known man made hybrid animal. It was likely invented in ancient times in what is now Turkey.

100 CE

A.D. 100: The first insecticide is produced in China from powdered chrysanthemums.

1856

Gregor Mendel experimented on pea plants to describe the relationship between traits in successive generations of plants known as the Laws of Inheritance.

1796

English surgeon Edward Jenner pioneers vaccination, inoculating a child with a viral smallpox vaccine.

1870

Breeders crossbreed cotton, developing hundreds of varieties with superior qualities.

1870-1910

Luther Burbank develops over 800 new stains of fruit, vegetables and flowers. His blight resistant potato is heavily planted in Ireland, ending the potato famine.

1928

Alexander Fleming discovered 'penicillin' from the mold Penicillium notatum, which could be used against many infectious diseases.

1942

Andrew Moyer develops a technique of mass producing penicillin by feeding cantaloupe mold

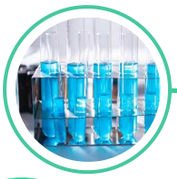
Modern Biotechnology
Post - 1950

Exploring biotechnology in the realm of history

At its simplest, biotechnology is technology based on biology - biotechnology harnesses cellular processes to develop technologies and products that help improve our lives and the health of our planet. Humans have been manipulating biological processes for thousands of years.
Here is an overview of the evolution of biotechnology:

1943

Oswald Theodore Avery isolates pure DNA.



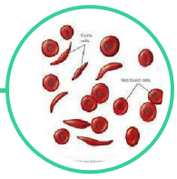
1953

James Watson and Francis Crick are the first to describe the double helix structure of DNA.

1955

First use of mammalian cells (monkey kidney cells) to produce the Polio vaccine.

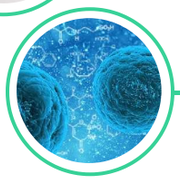
1957



Scientists prove that sickle-cell anemia occurs due to a change in a single amino acid in hemoglobin cells.

1961

Francois Jacob and Jacques Monod discover the role of mRNA in transferring genetic information from the nucleus to the cytoplasm.



1968

Nirenberg and Khorana decipher the genetic code for all 20 amino acids and conclude that the genetic code is universal among all living things.

1970

Werner Arber discovers restriction enzymes in bacteria that cut virus DNA. These enzymes are widely used in modern biotechnologies.

1973

The first recombinant DNA technology developed by Stanley Cohen and Herbert Boyer. They inserted a gene from an African clawed toad into bacteria.

1976

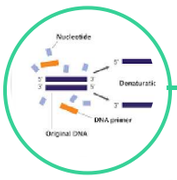
The nucleotide sequence for a specific gene is determined for the first time.

1977

Recombinant *E. coli* bacteria used to mass produce human insulin.

1983

Polymerase Chain Reaction (PCR), a technique to make multiple copies of DNA sequences is invented.



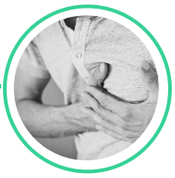
1984

Genetic fingerprinting is discovered and is used today to establish family relationships and identify criminals.

1986

The first genetically modified tobacco plants are grown outside in a field.

1987



Genetically engineered plasminogen activator is approved to treat heart attacks.

1989

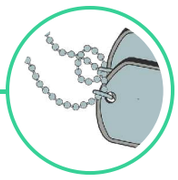
Oil-eating bacteria are used to clean up the Exxon Valdez oil spill.

1990

Recombinant Chymosin enzyme used in cheese making becomes one of the first food products to be manufactured using *E. coli.*

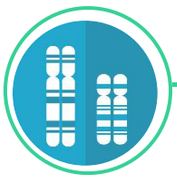
1992

US Army collects blood and tissue samples for genetic dog tag program aimed to improve identification of soldiers killed in combat



1993

A rough map of all 23 human chromosomes is completed.



1995

Haemophilus influenzae is the first living organism to be fully genome sequenced.

1997

Dolly the sheep, the first cloned mammal is produced.

1998

Human skin cells produced for the first time in a lab.

2001

Science and *Nature* magazines publish the human genome sequence, making it possible for scientists all over the world to begin researching new treatments for diseases that have genetic origins, such as cancer, heart disease, Parkinson's and Alzheimer's.

2005

The billionth biotech acre is planted by one of 8.5 million farmers in one of 21 countries.

2007

Recombinant DNA technology to produce the vaccine against human papillomavirus, cause of cervical cancer. Skin cells are also used to create embryonic stem cells.

2010

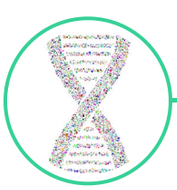
First self-replicating fully synthetic bacteria cell is created.

2011

Anti-HIV biotech medicine produced from genetically modified tobacco at a far cheaper price allowing wider access in poorer countries.

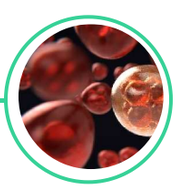
2012

CRISPR used to find specific DNA sequence and edit genes using techniques that are quicker and cheaper.



2012

Scientists report, for the first time, the use of the CRISPR technology to edit human genes to treat cancer patients with whom standard treatments were not successful.

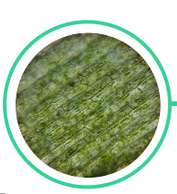


2013

The first bionic eye helps people recover partial sight.

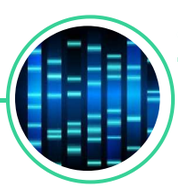
2020

Researchers report to have developed artificial chloroplasts – the photosynthetic structures inside plant cells.



2021

Scientists demonstrate a "Trojan horse" designer-nanoparticle that makes blood cells eat away – from the inside out – portions of atherosclerotic plaque that cause heart attacks



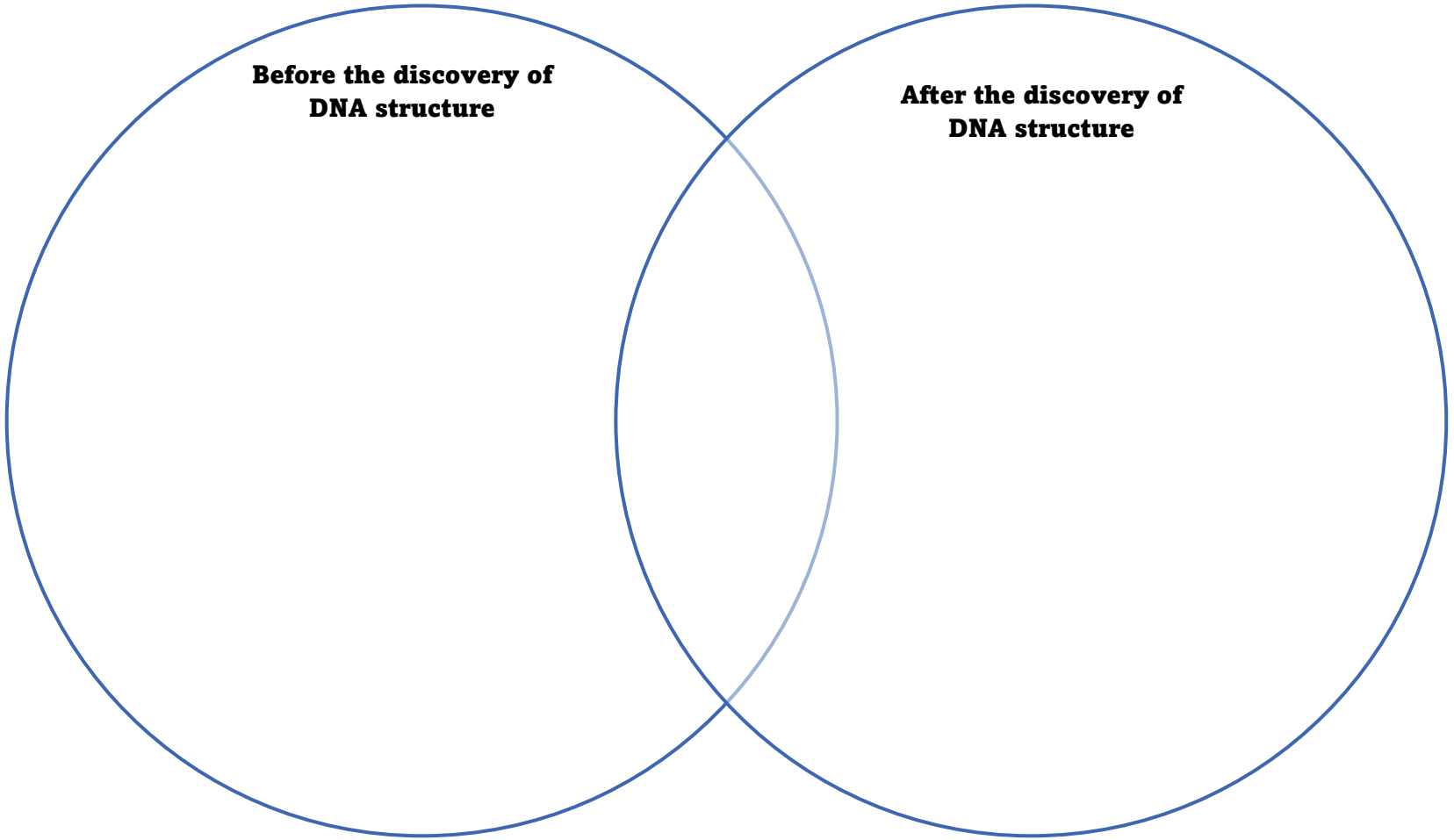
2022

The first successful xenogeneic heart transplant, from a genetically modified pig to a human patient, is reported.

2022

Microbiologists demonstrate an individually adjusted phage-antibiotic combination as an antimicrobial resistance treatment.

Comparing biotechnology before and after the discovery of DNA



Applications of Biotechnologies Note-making Scaffold

Use the following scaffold to make notes about three different applications of biotechnologies to genetically modify organisms. Examples of biotechnologies can be found on the biotechnology timelines provided in the previous learning activity and research resources are in the LabXchange pathway link below or other resources of your choosing.

https://www.labxchange.org/library/pathway/lx-pathway:adfaa302-f85d-4e0c-bd69-55e49600e298

Name of the biotechnology that genetically modifies organisms	
What problem is the biotechnology solving?	
How does the biotechnology work?	
How has an understanding of the structure of DNA lead to the development of this technology?	
What concepts do you need to clarify? **Or** **What questions came to mind while you were researching this biotechnology?**	

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How has an understanding of the structure of DNA lead to the development of this technology?	
What concepts do you need to clarify? **Or** **What questions came to mind while you were researching this biotechnology?**	

Extended Response Scaffold and Marking Guideline

Assess the impact of the discovery and understandings of the structure of DNA on the development of biotechnologies to genetically modify organisms.

Use the following scaffold to help you plan a response the extended response question above. Draw on examples and scientific knowledge gained throughout the AMGEN Biotech Experience. Write your final assessment in a separate document. You are then to self/peer mark your responses with the provided marking scheme and give yourself/peer warm and cold feedback.

Thinking /processing	Assessment of impact of scientific theories, laws and models on of the development of technology
Technologies prior to the scientific prinicple, theory, model or law → •Describe examples of technologies prior to discovery **Scientific priniples, theory, law, model** → •Describe the development of the scientific principle theory, law or model **Contribution to the technology** → •Explain the application of the scientific principles, theory, law or model in the development of new technologies **Make a Judgement** → •For example, the scientific principle has enhanced the development of this technology which has improved the.....	

Marking Scheme: Impact of scientific understanding on technology
7-8 Marks: Provides detailed characteristics and features of examples of biotechnologies prior to the discovery of the structure of DNA. Provides detailed characteristics and features of the development of understanding the structure of DNA Make the relationships evident between the understanding the structure of DNA and the development of two or more biotechnologies to genetically modify organism Makes a relevant judgement about the impact of the scientific theory, model, and/or law on the development of this technology.
4-6 Marks: Identifies examples of biotechnologies prior to the discovery of the structure of DNA. Provides characteristics and features of the development of understanding the structure of DNA Provides examples of biotechnologies to genetically modify organisms developed using an understanding of the structure of DNA Makes a judgement about the impact of the scientific theory, model, and/or law on the development of this technology.
2 - 3 Marks: Identifies examples of biotechnologies prior to the discovery of the structure of DNA. Identifies the development of understanding the structure of DNA Identifies an example of biotechnologies to genetically modify organisms
1 Mark: Provides some relevant information