AOHS Biotechnology

Lesson 6

Working with DNA in the Laboratory

Student Resources

Resource	Description
Student Resource 6.1	Lab Procedure: DNA Extraction from Cheek Cells
Student Resource 6.2	Note-Taking Guide: Working with DNA in the Laboratory
Student Resource 6.3	Reading: Working with DNA in the Laboratory
Student Resource 6.4	Lab Procedure: Using a Micropipet
Student Resource 6.5	Lab Procedure: Micropipet Battleship
Student Resource 6.6	Lab Procedure: Making and Loading Gels
Student Resource 6.7	Reading: Forensic DNA
Student Resource 6.8	Lab Procedure: Forensic DNA Analysis
Student Resource 6.9	Peer Review: Forensic DNA Lab Report
Student Resource 6.10	Assessment Criteria: Lab Notebook

Student Resource 6.1

Lab Procedure: DNA Extraction from Cheek Cells

*Student Name:*_____ *Date:*_____

Directions: In this exercise you will observe your own DNA by extracting it from your check cells. You will use the simple method of DNA extraction and you will explain the rationale of each step. You will be able to explain why DNA extraction is important to scientists. Through this exercise you will see how DNA is in every cell in the body and can be extracted easily.

Safety/Precautions

- Do not handle anyone's materials except your own. Immediately dispose of plastic cups.
- Do not ingest (eat or drink) any materials used in this lab except water that is supplied.

Materials

For each student:

- 2 teaspoons (10 ml) 0.9% salt water (2 teaspoons table salt in one quart/liter of water)
- Disposable paper or plastic cup
- Large test tube (or any clear tube that can be sealed with a rubber or cork stopper or preferably parafilm for safety)
- 1 teaspoon (5 ml) 25% mild detergent or dishwashing soap (e.g., Woolite or Palmolive,1 volume detergent or soap + 3 volumes water)
- 2 teaspoons (10 ml) 95% ethanol, chilled on ice
- Small test tube with seal

Introduction

The cell itself and the nucleus within the cell are both surrounded by membranes made primarily of phospholipids. Detergent destroys the membranes by emulsifying lipids in water and thereby exposes the contents of the cell and nucleus, including the DNA.

Saltwater helps remove cells from your cheeks and also makes the watery solution denser. This increased density will facilitate the separation of the DNA strands into the alcohol.

Alcohol is less dense than water, so it floats on top of the water. Most of the cellular components are "heavy" (dense) enough to remain in the watery solution at the bottom of the test tube. DNA, however, is less dense than either the water or the alcohol, so it floats to the surface of the alcohol. DNA is soluble in water but is not soluble in alcohol. Once the DNA floats up into the alcohol it *precipitates*, which means that it comes out of solution. This method separates DNA from the other parts of the cell.

DNA is exceedingly thin (0.0000002 mm), but because it clumps together it is visible to the eye. This is similar to cells, which individually must be seen with a microscope but when clumped together in multicellular organisms are visible to the eye.

Lab Instructions

This extraction will work best if you have not just recently eaten or chewed gum.

1. Add 5 mL of the soap solution to a test tube.

2. Violently swish the saltwater in your mouth (do not swallow) for 30 seconds, making sure toyou're your tongue along your cheeks.

3. Carefully spit the water mixture back into the plastic cup.

4. Pour your spit into the test tube until is about half full.

5. Place the cap back on the test tube, gently rocking the tube back and forth for one to two minutes. (Make sure to place one finger on the lid, with another holding the bottom of the tube to prevent the lid from coming off.)

6. Add enough ice cold ethyl alcohol to almost fill the test tube. After this DO NOT tip, shake, or mix the test tube, or you may not see DNA.

7. If you look at the line of separation between the layer of water and the layer of alcohol (the interface), you will start to see bubbles attached, with tiny hair-like white strings rising through the alcohol. These strings are your DNA.

8. To collect the DNA by spooling, carefully place a glass stir rod though the alcohol layer until it touches the bottom of the tube. Slowly spin the rod between the fingers, while watching the interface between the two layers. If enough DNA is present, it will clump together at the interface between layers to form a milky translucent mass. Spin the rod to wrap the DNA around it (that is the spooling part) and pull it out of the tube. The DNA can be transferred to another tube of pure alcohol for storage or further analysis.

Questions

1. What was the purpose of the detergent?

2. Was the DNA you extracted from one cell or from many cells?

3. Describe the appearance of the DNA.

4. Why is it important that scientists can extract DNA from cells?

Student Resource 6.2

Note-Taking Guide: Working with DNA in the Laboratory

*Student Name:*_____ *Date:*_____

Directions: Read the four questions in the table. As you view the presentation on working with DNA in the laboratory, take notes that answer the questions.

Questions	Answers from Presentation
1. What are the basic structures contained in the DNA molecule?	
2. What are the names of the four nitrogen-containing nucleobases?	

Questions	Answers from Presentation
3. What are some biotechnology fields that use DNA analyses?	
4. What is gel electrophoresis? Describe the steps in a successful DNA profile using electrophoresis.	

Student Resource 6.3

Reading: Working with DNA in the Laboratory

AOHS

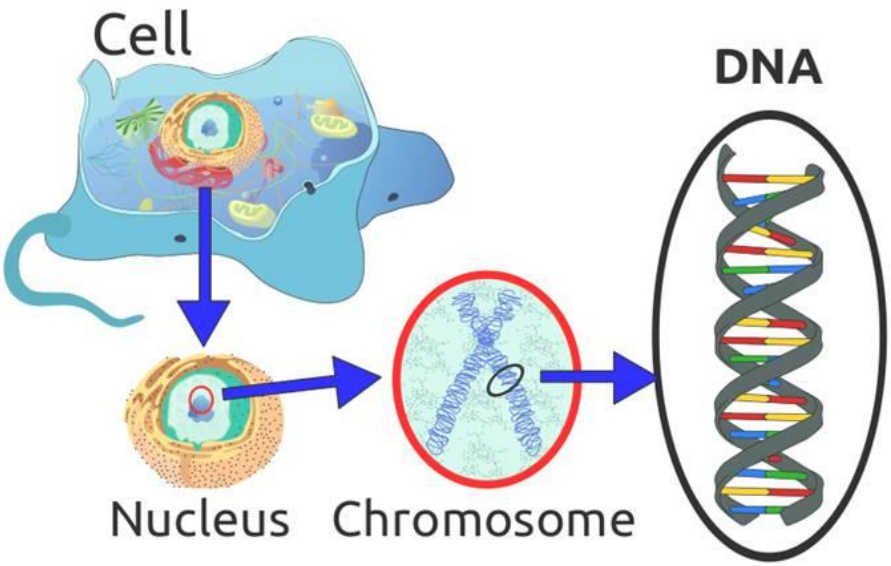
Biotechnology

Unit 2, Lesson 6

Working with DNA in the Laboratory

Today, you are going to learn about working with DNA in the laboratory. You will learn about the various scientific laboratory methods used to analyze DNA for genetics, criminal investigation, and medical reasons. We will also study careers in biotechnology that work with DNA.

DNA and proteins are key molecules of the cell nucleus



Why do you think a DNA molecule consists of two strands?

Let's start with a review of the basics of the structure of DNA. Deoxyribonucleic acid (DNA) is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses. DNA is a nucleic acid; along with proteins and carbohydrates, nucleic acids compose the three major macromolecules essential for all known forms of life.

Why does a DNA molecule consist of two strands? The primary function of DNA is to store and transmit genetic information. To accomplish this function, DNA must have two properties. It must be chemically stable to reduce the possibility of damage. DNA must also be capable of copying the information it contains. The two-stranded structure of DNA gives it both of these properties.

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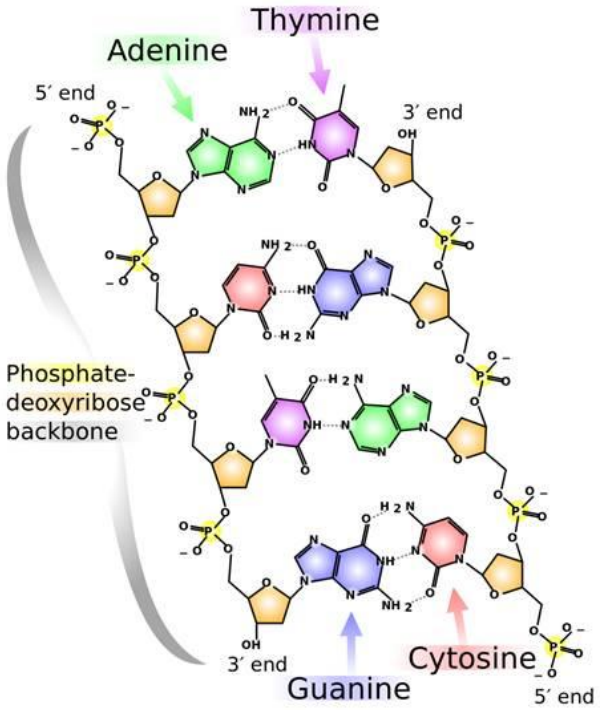
DNA is a long polymer made from repeating units called nucleotides

Nucleotides consist of three components:

- Nitrogenous base
- Deoxyribose and ribose
- One or more phosphate groups

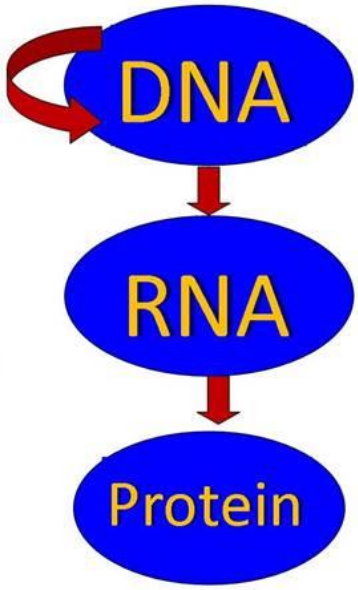


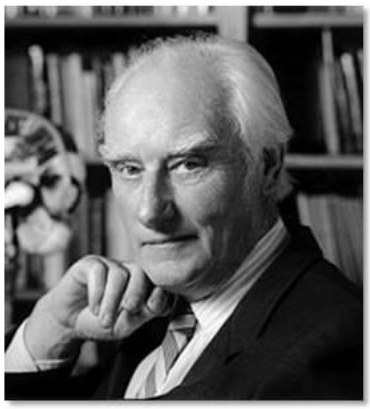
Most DNA molecules consist of two biopolymer strands coiled around each other to form a double helix. The two DNA strands are known as polynucleotides since they are composed of simpler units called nucleotides. Each nucleotide is composed of a nitrogen-containing nucleobase—either guanine (G), adenine (A), thymine (T), or cytosine (C)—as well as a monosaccharide sugar called deoxyribose and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. According to base pairing rules (A with T and C with G), hydrogen bonds bind the nitrogenous bases of the two separate polynucleotide strands to make double-stranded DNA.

Image retrieved from http://en.wikipedia.org/wiki/Structure_of_DNA#mediaviewer/File:DNA_chemical_structure.svg on 8/5/14. Courtesy of Madeleine Price Ball.

Francis Crick first stated the important Central Dogma in biotechnology in 1958

The Central Dogma explains how genetic information flows within a biological system.



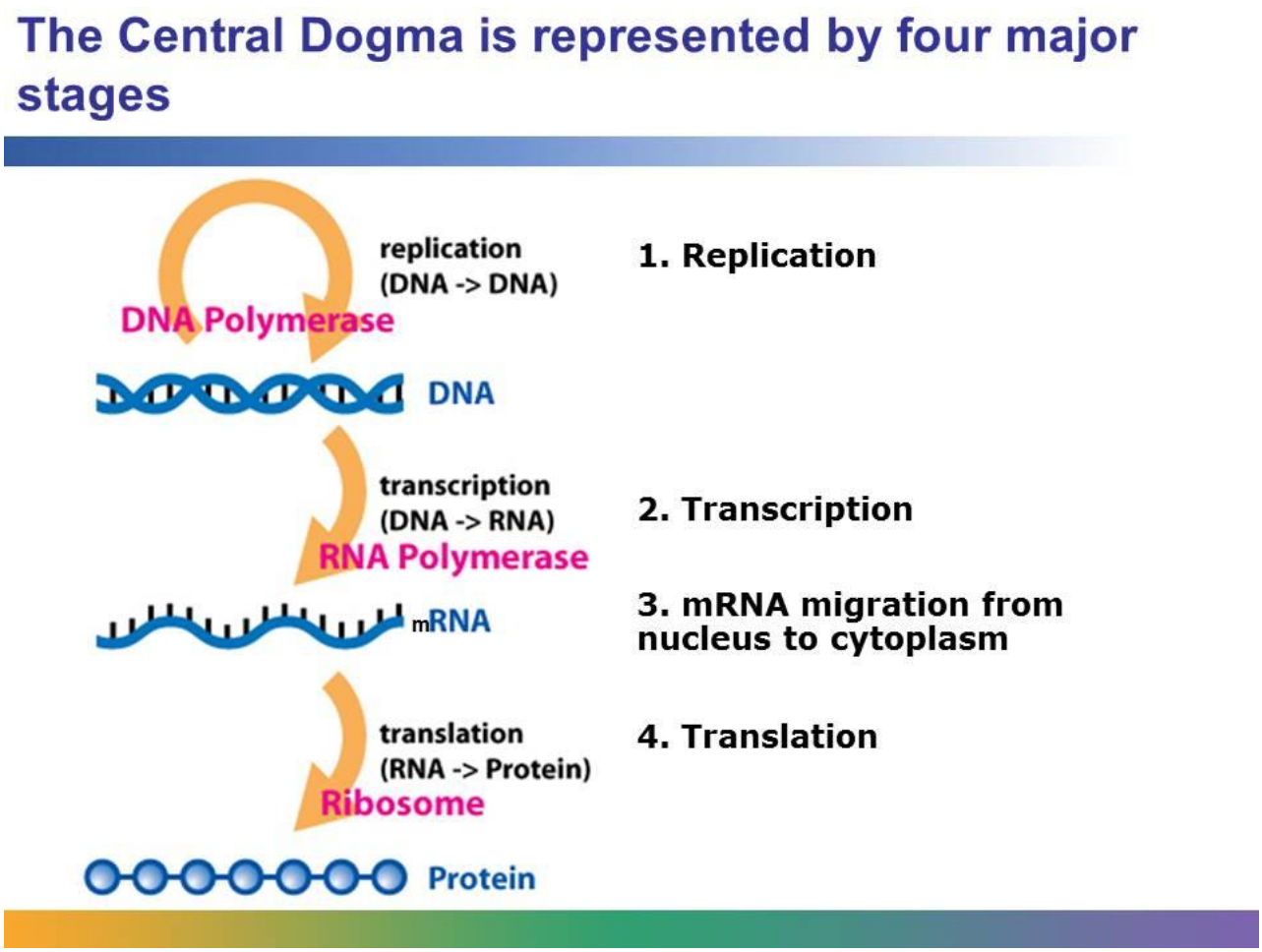


Francis Crick

Francis Crick was an important theoretical molecular biologist and played a crucial role in research related to revealing the genetic code. He is widely known for use of the term "central dogma" and having solved the structure of DNA along with James Watson in the 1950s.

The Central Dogma is illustrated above. It has also been described as "DNA makes RNA makes protein". However, this statement is a little bit oversimplified. Crick's Central Dogma does not say that information cannot flow in reverse from RNA to DNA. It does rule out the flow from protein to RNA or DNA. In other words, the central dogma of molecular biology states that such information cannot be transferred back from protein to either protein or nucleic acid.

Photo by Marc Lieberman. Retrieved from http://commons.wikimedia.org/wiki/File:Francis_Crick_crop.jpg on 8/5/14 and reproduced under the CC Attribution 2.5 Generic license (http://creativecommons.org/licenses/by/2.5/deed.en). Graphic image reproduced here under fair-use guidelines of Title 17, US Code.



Transcription of DNA to RNA to protein: This dogma forms the backbone of molecular biology and is represented by four major stages.

1. The DNA replicates its information in a process that involves many enzymes: replication.

2. The DNA codes for the production of messenger RNA (mRNA) during transcription.

3. In eucaryotic cells, the mRNA is processed (essentially by splicing) and migrates from the nucleus to the cytoplasm.

4. Messenger RNA carries coded information to ribosomes. The ribosomes "read" this information and use it for protein synthesis. This process is called translation.

Proteins do not code for the production of protein, RNA, or DNA. They are involved in almost all biological activities, structural or enzymatic.

Source: http://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/central_dogma.html

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DNA analysis has many uses in biotechnology

- Genetic engineering
- Forensics
- Bioinformatics
- DNA nanotechology
- Molecular anthropology

Genetic engineering, also called genetic modification, is the direct manipulation of an organism's genome using biotechnology.

Forensic DNA profiling (also called DNA testing, DNA typing, or genetic fingerprinting) is a technique used by forensic scientists to help identify individuals by their respective DNA profiles.

Bioinformatics is an interdisciplinary scientific field that develops methods and software tools for storing, retrieving, organizing, and analyzing biological data.

DNA nanotechnology is the design and manufacture of artificial nucleic acid structures for technological uses.

Molecular anthropology is a field of anthropology in which molecular analysis is used to determine evolutionary links between ancient and modern human populations, as well as between contemporary species.

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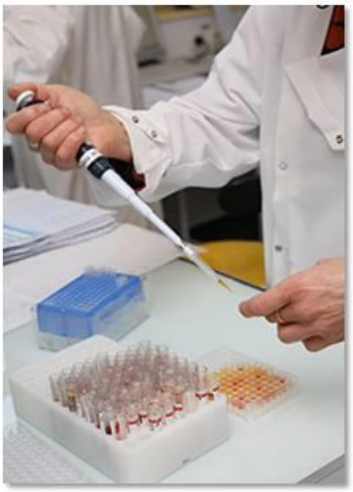
Biotechnologists extract and analyze DNA for many scientific applications

Biotechnologists working with ancient samples and conducting DNA analysis on Neanderthal bones.



How do you think analyzing ancient human bones contributes to our knowledge today?

DNA is extracted from human cells for a variety of scientific reasons. With a pure sample of DNA you can test a newborn for a genetic disease, analyze forensic evidence, learn about ancient peoples, or study a gene involved in cancer.

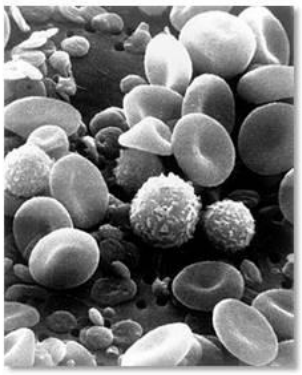
Biotechnologists often work in a clean room to avoid contamination of the lab samples, like this Neanderthal bone sample undergoing analysis.

Source: http://learn.genetics.utah.edu/content/labs/extraction/

Image (left) by Max Planck Institute for Evolutionary Anthropology. Retrieved from

http://commons.wikimedia.org/wiki/File%3ANeanderthal_DNA_extraction.jpg; on 8/5/14. Image (right) retrieved from Wikimedia Commons.

DNA extraction is a routine procedure in molecular biology laboratories



White blood cells

DNA can be extracted from many types of cells

Since DNA is the blueprint for life, every living thing contains DNA. DNA isolation or extraction is one of the most basic and essential techniques in the study of DNA. The extraction of DNA from cells and its purification are of primary importance to the field of biotechnology and forensics. Extraction and purification of DNA allows scientists to detect genetic disorders, produce DNA fingerprints of individuals, and even create genetically engineered organisms that can produce beneficial products such as insulin, antibiotics, and hormones.

DNA can be extracted from many types of cells. Individual strands of DNA are too small to be visible to the eye. One million threads of DNA fit onto the period at the end of a sentence. We are able to see DNA in a lab extraction because there are so many of them clumped together.

White blood cells photograph by Bruce Wetzel. Retrieved from http://en.wikipedia.org/wiki/White_blood_cell#mediaviewer/File:SEM_blood_cells.jpg on 8/5/14. Strawberry by Rlaferla. Retrieved from http://commons.wikimedia.org/wiki/File:PerfectStrawberry.jpg , reproduced under the GNU FDL. Goat by Armin Kubelbeck. Retrieved from http://commons.wikimedia.org/wiki/File:Hausziege_04.jpg . Reproduced under CC BY_SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0/deed.en).

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Forensic scientists use DNA profiling to identify individuals

Forensic DNA Analysis relies on two basic facts:

1. Every individual (except identical twins) has a unique DNA sequence.
2. Each cell in an individual contains the same DNA sequence.

DNA profiling is used in parental testing and criminal investigation.

DNA profiling is a technique used by forensic scientists to help identify individuals by their respective DNA profiles.

Forensic scientists can use DNA in blood, semen, skin, saliva, or hair found at a crime scene to identify a matching DNA of an individual. This process is formally termed DNA profiling but may also be called genetic fingerprinting. DNA profiling is often used in parental testing and criminal investigations.

Although 99.9% of human DNA sequences are the same in every person, enough of the DNA is different that it is possible to distinguish one individual from another, unless they are identical twins.

Photo by Eddy Van 3000. Retrieved from http://commons.wikimedia.org/wiki/Category:Human_twins#mediaviewer/File:Redhead_twins.jpg on 8/5/14. Reproduced under CC BY-SA 3.0 license (http://creativecommons.org/licenses/by-sa/2.0/deed.en).

DNA profiling can identify an individual based on his or her DNA sequence

DNA analysis can help:

- Solve crimes
- Identify human remains
  - Missing persons
  - Disasters
- Determine relationships between family members like paternity and with child abductions

DNA profiling (also called DNA testing, DNA typing, or genetic fingerprinting) is a technique used by forensic scientists to help identify individuals by their respective DNA profiles. DNA profiles are encrypted sets of letters that reflect a person's DNA makeup, which can also be used as the person's identifier. DNA profiling should not be confused with full genome sequencing.

The DNA profiling technique was first reported in 1986 by Sir Alec Jeffreys at the University of Leicester in England, United Kingdom, and is now the basis of several national DNA databases. Dr. Jeffreys' genetic fingerprinting was made commercially available in 1987.

Image retrieved from http://www.nlm.nih.gov/visibleproofs/media/detailed/iii_d_220.jpg on 8/5/14. Reproduced here under fair-use guidelines of Title 17, US Code.

Human DNA evidence can be found in many places

We leave cells behinds us wherever we go. DNA can be isolated from skin, blood, hair, semen, urine, and saliva.

Can you think of any other places where DNA might be deposited if you are the investigator looking for evidence?

DNA can be collected from a sample of blood, saliva, semen, or other appropriate fluid or tissue from personal items such as a toothbrush or razor. DNA can also be obtained from stored samples such as banked sperm or biopsy tissue. Samples obtained from blood relatives can provide an indication of an individual's profile, as could human remains that had been previously profiled.

A reference sample is then analyzed to create the individual's DNA profile. The DNA profile is compared against another sample to determine whether there is a genetic match.

Image retrieved from http://dnaproject.co.za/blog/dna-project-bacsa-cpfs-saps-collaborate-for-greater-crime-scene-awareness on 8/5/14. Reproduced here under fair-use guidelines of Title 17, US Code.

DNA fingerprinting establishes a link between biological evidence and a suspect

DNA fingerprint analysis from the crime scene and the DNA profile of the suspects.

In what other situations could this DNA information be used outside of a crime investigation?

DNA fingerprinting is a laboratory technique used to establish a link between biological evidence and a suspect in a criminal investigation. A DNA sample taken from a crime scene is compared with a DNA sample from a suspect. If the two DNA profiles are a match, then the evidence came from that suspect. Conversely, if the two DNA profiles do not match, then the evidence cannot have come from the suspect. DNA fingerprinting is also used to establish paternity.

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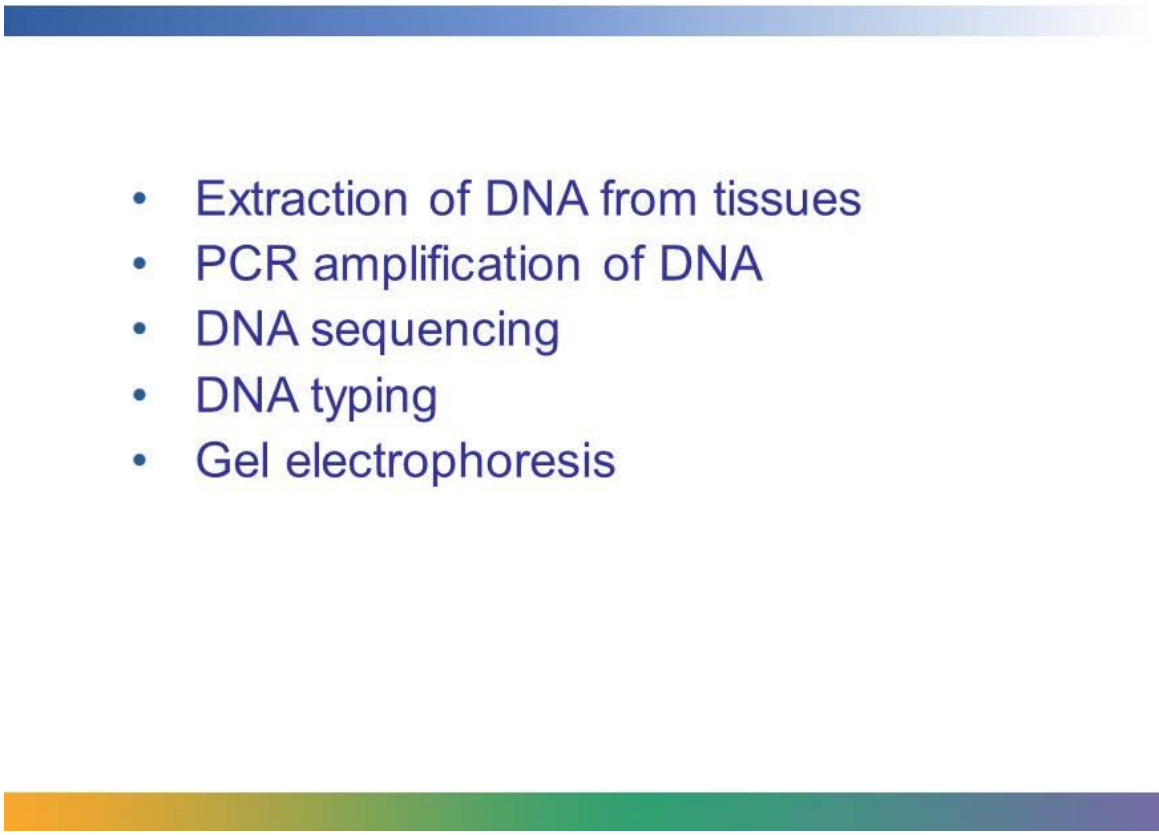
Standard DNA lab methods



Obtaining high-quality, intact DNA is often the first and most critical step in many fundamental molecular biology applications, such as DNA cloning, sequencing, PCR, and electrophoresis. Isolating intact total DNA from tissue samples varies in difficulty with the physical and biochemical nature of the tissue.

The following slides will examine these standard DNA Lab methods:

- Extraction of DNA from tissues
- PCR amplification of DNA
- DNA sequencing
- DNA typing
- Gel electrophoresis

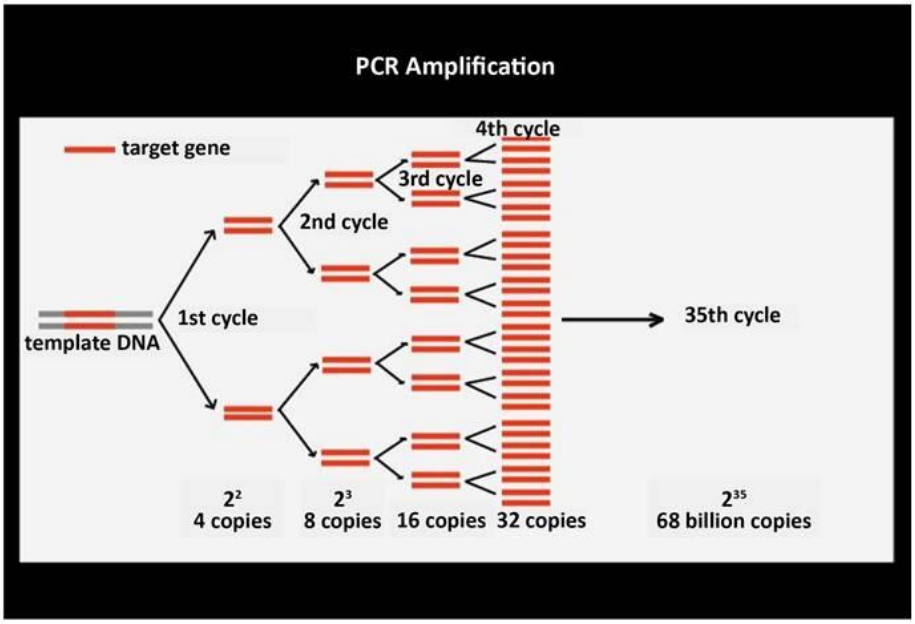
Extraction of DNA is a common lab procedure

- Breaking the cells open, commonly referred to as cell lysis, to expose the DNA within
- Removing membrane lipids by adding a detergent, which also serves in cell lysis
- Removing proteins by adding a protease
- Removing RNA by adding an RNase
- DNA purification from detergents, proteins, salts, and reagents used during cell lysis step

The extraction of DNA from tissues or cells is a common procedure in a biotechnology laboratory. Biotechnologists work with DNA in the laboratory with versions of this procedure:

- Breaking the cells open, commonly referred to as cell disruption or cell lysis, to expose the DNA within. This is commonly achieved by chemical and physical methods-blending, grinding, or sonicating the sample.
- Removing membrane lipids by adding a detergent or surfactants, which also serves in cell lysis.
- Removing proteins by adding a protease (optional but often done).
- Removing RNA by adding an RNase (almost always done).
- DNA purification from detergents, proteins, salts, and reagents used during cell lysis step.

Polymerase chain reaction (PCR) makes many copies of a sequence of DNA



Why do you think a biotechnologist working in a laboratory needs to make many copies of extracted DNA?

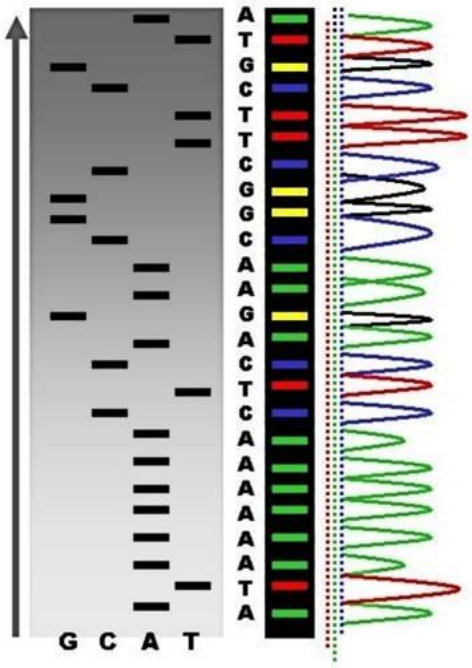
Polymerase chain reaction (PCR) is a way to make many copies of a sequence of DNA. This is done in a lab, using an enzyme called DNA polymerase. It is called chain reaction because the result of one cycle is used immediately for the next cycle. This allows exponential growth to happen.

PCR is extensively used in the analysis of clinical specimens for the presence of infectious agents, including HIV, hepatitis, human papillomavirus, malaria, and anthrax.

Biotechnologists can often extract only small amounts of the DNA they are interested in from a specimen. These amounts are usually too little to be useful, and the lab scientist will use PCR to make enough copies to start experimentation. For this reason, it is one of the most common techniques used in genetics labs around the world, making it useful in experiments on many things, including gene therapy, infectious diseases, and forensics.

Image retrieved from http://www.paulvanouse.com/dwpcr.html on 8/5/14. Reproduced here under fair-use guidelines of Title 17, US Code, courtesy Paul Vanouse.

DNA sequencing determines the precise order of nucleotides in a DNA molecule



This is an example of the results of automated chain-termination DNA sequencing.

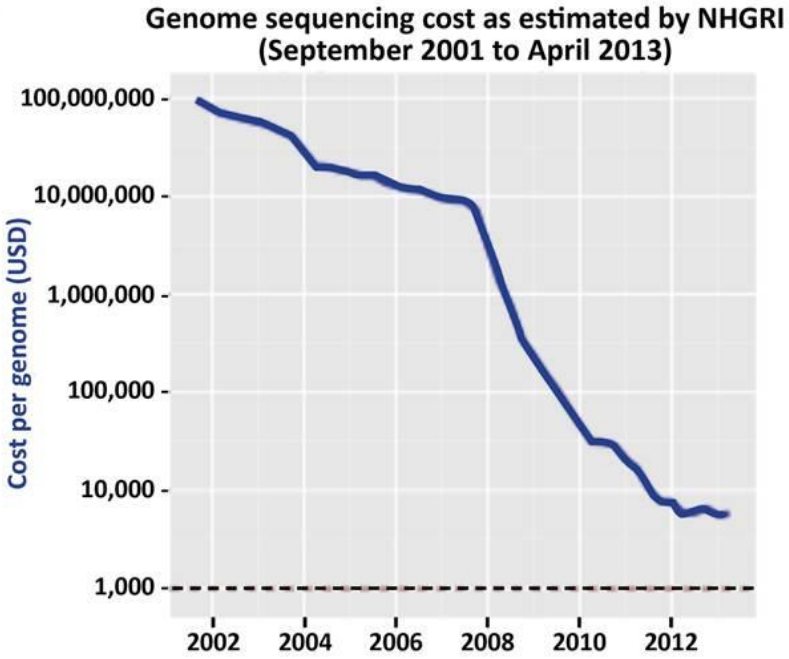
DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA. New and faster DNA sequencing methods have greatly accelerated biological and medical research and discovery.

The rapid speed of sequencing attained with modern DNA sequencing technology has been instrumental in the sequencing of complete DNA sequences, or genomes of numerous types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species.

Image retrieved from http://en.wikipedia.org/wiki/DNA_sequencing#mediaviewer/File:Radioactive_Fluorescent_Seq.jpg on 8/5/14. Reproduced under CC-BY-SA-2.5,2.0,1.0, GNU Free Documentaton License.

New technologies make DNA sequencing less expensive and more accessible

DNA sequencer

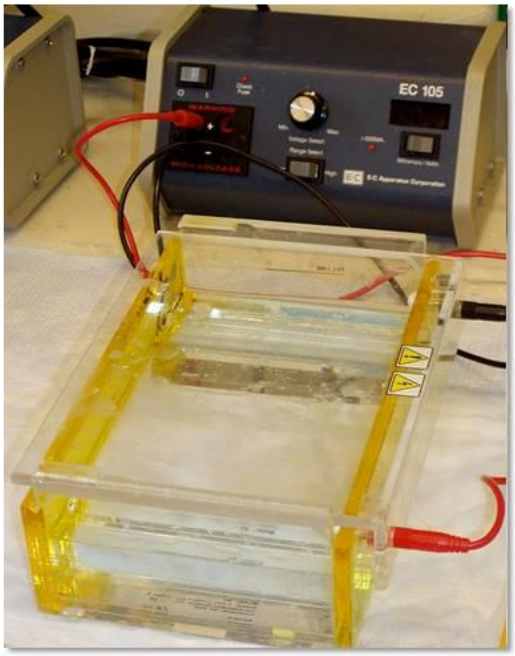


For many years, the National Human Genome Research Institute (NHGRI) has tracked the costs associated with DNA sequencing performed at the sequencing centers funded by the Institute. DNA sequencing technologies are less expensive today.

Photo by Konrad Forstner. Retrieved from http://en.wikipedia.org/wiki/DNA_sequencing#mediaviewer/File:Illumina_MiSeq_sequencer.jpg on 8/5/14. Graph retrieved from http://www.genome.gov/sequencingcosts and reproduced here under fair-use guidelines of Title 17, US Code (data from the NHGRI Genome Sequencing Program).

Short tandem repeat (STR) analysis is a molecular biology method used to compare specific loci (locations) on DNA from two or more samples. STR analysis measures the exact number of repeating units. This method differs from restriction fragment length polymorphism analysis (RFLP) because STR analysis does not cut the DNA with restriction enzymes. Instead, probes are attached to desired regions on the DNA, and a polymerase chain reaction (PCR) is used to discover the lengths of the short tandem repeats.

Gel electrophoresis is a laboratory method used to solve DNA identity issues



Gel electrophoresis apparatus

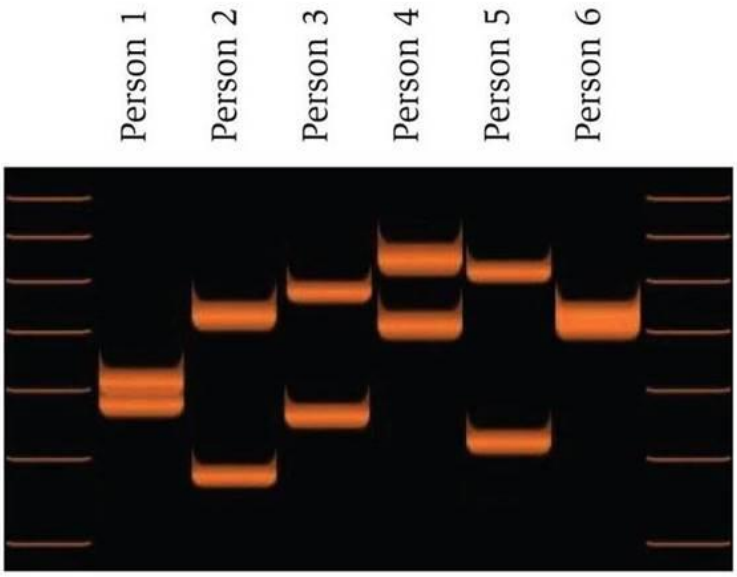
An agarose gel is placed in this buffer-filled box and an electrical field is applied via the power supply to the rear. The negative terminal is at the far end (black wire), so DNA migrates toward the anode (red wire).

Gel electrophoresis is a method for separating and analyzing macromolecules (DNA, RNA, and proteins) and their fragments, based on their size and charge.

Nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through a matrix of agarose or other substances. Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. This phenomenon is called sieving. Proteins are separated by charge in agarose because the pores of the gel are too large to sieve proteins. Gel electrophoresis can also be used for separation of nanoparticles.

Photo by Jeffrey M. Vinocur. Retrieved from http://en.wikipedia.org/wiki/Gel_electrophoresis#mediaviewer/File:Gel_electrophoresis_apparatus.JPG on 8/5/14 and reproduced under CC-BY-AS 3.0 license (http://creativecommons.org/licenses/by-sa/3.0/deed.en).

Results of gel electrophoresis with a DNA profiling example with six samples



Do you think that any of these DNA profiles could be the same person?

The process of DNA profiling with gel electrophoresis begins with a sample of an individual's DNA, typically called a reference sample. The most desirable method of collecting a reference sample is by using a buccal or cheek swab, because this reduces the possibility of contamination. A reference sample is then analyzed to create the individual's DNA profile using one of a number of techniques like we have just discussed. The DNA profile is then compared against another sample or samples from people to determine whether there is a genetic match. DNA profiling is like getting a fingerprint, and a person's DNA is unique with a few exceptions, such as identical twins.

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These scientists laid the foundation for all the possibilities for DNA work in the laboratory

Watson and Crick

Rosalind Franklin

Without their work, none of these technologies—and the discoveries these technologies permit—would have happened!

- Extraction of DNA from tissues
- PCR amplification of DNA
- DNA sequencing
- DNA typing
- Gel electrophoresis

All of these procedures for working with DNA in the laboratory were made possible by James Watson and Francis Crick's discovery of the DNA double helix structure. These two scientists combined theory, modeling, and experimental results to achieve their goal of understanding the microcellular structures in genetics. They won the 1962 Nobel Prize in Physiology or Medicine "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material." Rosalind Francis was the researcher who used a technique called X-ray crystallography to gather data about the structure of DNA that enabled Watson and Crick to make their famous discovery.

These scientists laid the foundation for all of the possibilities in the field of molecular biology and all of the new biotechnologists who will explore DNA in biological laboratories in the future.

Using DNA for research, analysis, or experimentation is an important part of biotechnology. During this lesson, you will develop some basic skills at working with DNA in a laboratory, too!

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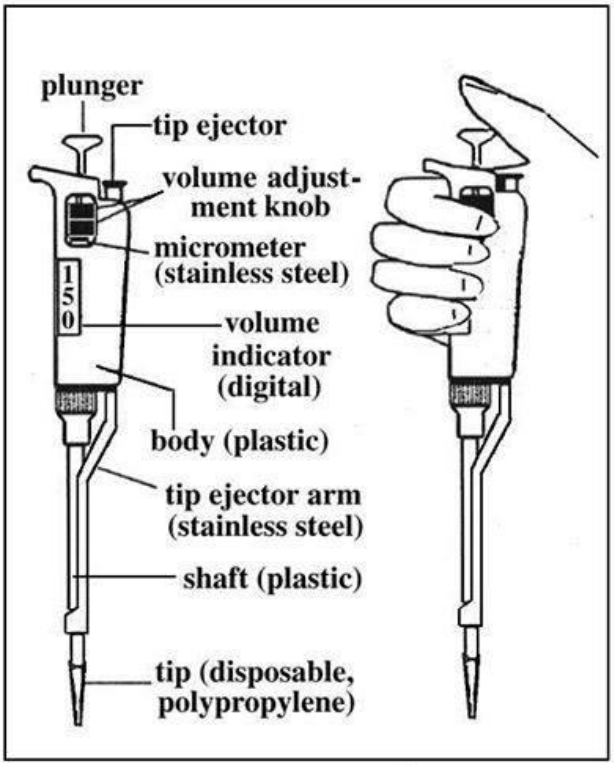
Student Resource 6.4

Lab Procedure: Using a Micropipet

*Student Name:*_____ *Date:*_____

Directions: Micropipets are the standard laboratory equipment used to measure and transfer small volumes of liquids. In this lesson, micropipets are also referred to as pipets. You will use them throughout this course and in courses that you take in the future, and it is therefore essential that you master their use. You will develop the skills necessary to accurately use a micropipet, understand how to accurately measure volumes in microliters (ul), and practice metric conversions. You will practice using micropipets with liquids of differing viscosity, adjusting the pipet for appropriate volumes, and checking the accuracy of their measurements.

Parts of a Micropipet



Three Sizes of Micropipets

The micropipets in this laboratory come in three different sizes, each of which measures a different range of volumes. The three sizes are P20, P200, and P1000. These sizes are noted on the top of the plunger button.

Size of micropipet	Range of volumes measured
P20	0.5–20 µl
P200	20–200 µl
P1000	100–1000 µl

Materials for Micropipet Technique Practice

At your lab station:

- Two 200 µl pipette tips
- 20 µl pipette
- Beaker containing red liquid
- Unlabeled empty microtubes
- Samples A–C in labeled microtubes
- Parafilm
- Microtube rack
- Waste container
- Sharpie

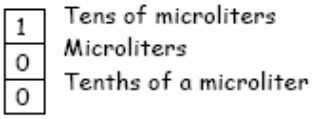
At the common station:

- Scale
- 1000 µl pipet tips
- 1000 µl pipet
- Beakers of Samples A–C
- Unlabeled empty microtubes

Procedure

Part I: Using a 20 µl Pipet

1. Obtain a 20 ul micropipet, and adjust the reading to 10 ul. Remember to rotate the dial until the volume window looks like this:



2. Press the micropipet into one of the tips. Press firmly enough to seal the tip, but not so hard that you damage the pipet. Steady the hand that is holding the micropipette.

3. Depress the plunger to the first stop position. Hold the micropipet vertically (straight up and down), immerse the tip approximately 2 mm into the beaker containing the red liquid. Release the pressure, allowing the plunger to move up slowly. This will pull the liquid into the tip (make sure there are not any bubbles—this distorts your measurement).

4. Remove the tip from the liquid and release the sample into the waste container by pushing the plunger to the **first** stop position.

5. Wait about one second and release any remaining liquid in the tip by pressing the plunger to the **second** stop position (it should be all the way down now).

6. Leaving the plunger down, remove the micropipet from the container and then slowly allow the plunger to return to the up position.

7. Dispose of the tip by pushing down on the eject button while holding the micropipet over the waste container.

Part II: Pipetting Practice

8. Label three tubes microtubes 1, 2, and 3 with a Sharpie. Initial and date your tubes.

9. Add the amount of samples A, B, and C to tubes as shown below:

Tube	Sample A	Sample B	Sample C	Total Volume
1	4 µl	5 µl	0 µl	
2	6.5 µl	2.5 µl	10 µl	
3	3 µl	0 µl	2.5 µl	

10. Tap the tube on the table to pool the solution on the bottom. You can also hold the top of the tube in one hand and use the middle finger on your other hand to flick the bottom of the tube.

11. Add up the total volume of liquid in Tube 1. As a check of your technique, set the micropipet to that volume and withdraw all of the liquid in Tube 1. The contents should just fill the tip, with no airspace at the top, and no leftover liquid in the tube. Dispose of the liquid and the tip in the waste beaker.

12. Add up the total volume of Tube 2. Check your technique by setting the pipette to the correct volume and withdraw all the solution in Tube 2.

13. Add up the total volume of the liquid in Tube 3. Check your technique by setting the pipet to the correct volume and withdraw all the solution in Tube 3.

Part III: Drop Sizes

14. Take a piece of parafilm and lay it flat on your lab station. You will be mixing your samples here.

15. Place a tip on the 20 µl pipette.

16. Measure 6 µl of Sample A and place this on the parafilm.

17. Dispose of your used tip and get a fresh tip. Measure 6 µl of Sample B and mix this drop with Sample A.

18. Dispose of the used pipet tip.

19. Hand the pipet to your partner, who will repeat Steps 2–5, creating a new drop.

20. Compare the size of your drops.

Part IV: The 1000 µl Challenge: Your Skill Assessment

21. Go to the common station and wait for your teacher. While you are waiting, read the rest of these directions, but do not do anything until you are instructed to do so.

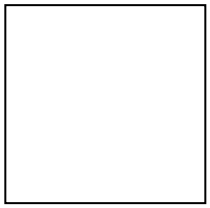
22. Pick up the 1000 µl micropipet and place a tip on it.

23. Measure the following volumes into one microtubes:

Tube	Sample A	Sample B	Sample C	Total
1	250 µl	300 µl	200 µl	

24. Calculate the total that should be in the tube.

25. Show your teacher your total to complete this assessment.



←Stamp Box

Student Resource 6.5

Lab Procedure: Micropipet Battleship

*Student Name:*_____ *Date:*_____

Directions: Today, you will play the game Battleship while practicing your micropipet skills. You and your partner will construct game boards in 96-well plates using uncolored water to represent blank ocean and blue-colored water to show where the ships are. You will be using the micropipets to fill each well, and you should be very comfortable with their use by the end of the activity.

How to Play

1. Review proper use and handling of the micropipets with your partner, then select a P100 micropipet by checking the range on the top of the plunger. What does 10/100 mean? What is the smallest volume this pipet should be dialed to? What is the largest volume this pipet should be used on? What are the first and second stops?

2. Set up a folder on top of the lab table between you and your partner to block each other's view. Using the outline of the 96-well plate below, map out where you will place your ships by putting *X*s in those squares. *DO NOT LET YOUR PARTNER SEE YOUR MAP!*

Your ships include:

- Two patrol boats (two wells each)
- Two destroyers (three wells each)
- One submarine (four wells)
- One aircraft carrier (five wells)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

3. Once all your ships are mapped, begin filling your 96-well plate with 80 µl of clear or blue water according to your map. Fill all the blue wells first, then eject the pipette tip into the trash. Put on a clean tip before switching to the clear water to fill all remaining wells.

4. When you and your partner are ready, you can begin taking turns calling out coordinates to see if you can hit and sink each other's ships.

5. When your partner calls a coordinate on your board, use the micropipet to add 50 µl of red water to each well that he or she calls. Tell your partner if it was a miss (there was no ship at that location) or if it was a hit (they successfully guessed the location of one of your ships). To sink a ship, they need to hit all the wells for that ship. The first player to sink all of the other's ships wins.

6. On the extra well plate outline below, keep track of the coordinates and the hits or misses that you have called.

Biotech Battleship

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Student Resource 6.6

Lab Procedure: Making and Loading Gels

Introduction

The purpose of this laboratory exercise is to introduce you to the method of gel electrophoresis by learning to mix and pour agarose gels of varying consistencies. In this laboratory exercise, you will predict how changing the percent agarose affects the outcome. You will also practice micropipetting skills.

The goal is to have you understand how gels are made for DNA separation and how altering the composition can affect the experimental parameters.

Safety Note

You should use gloves and goggles. Also use caution while heating and dissolving agarose in the TBE buffer. When heating and dissolving with a microwave oven or on a hot plate, always loosen the flask cap, use a hot hand protector, and use extreme caution when swirling hot mixture—never hold the flask near your face, because the superheated solution could boil over.

Materials

For each pair of students (note the gels prepared will NOT be used in any analysis):

- 2 grams of agarose
- 100 ml 1X Tris/Borate/EDTA (TBE) buffer
- Gel casting tray and comb
- High-walled container (e.g., plastic box, tray)
- Erlenmeyer flask
- Stir bar
- Food coloring (Sample Solution C)
- Gloves (one pair per student)
- Goggles (one per student)
- Masking tape
- Water
- 0–20 µl micropipet (P20) (for every three or four pairs)
- Hot/stir plate (for every three or four pairs) or microwave oven
- Balance (one for the class)

Lab Instructions

Prepare the Agarose Gel

1. With your partner, decide on a different percent agarose gel to make, in the range of 0.5% to 2%. Record this in your lab notebook and show the calculation you will use to make that percentage

gel. Since the percentage is by weight (and the buffer weighs approximately 1 mg/ml), a solution of 1.5 grams of agarose dissolved in 100 grams (e.g., 100 milliliters) of buffer will yield a 1.5% gel.

2. Predict how the speed of DNA through the gel will change as the percentage of agarose is increased.

3. Weigh out the proper amount of agarose on the balance and add it to 100 ml of TBE buffer.

4. Put the flask on the hot plate with a stir bar running slowly or use a microwave oven, paying attention to the safety note above. If you do not have a hot plate with stirring bar you may gently swirl the mixture, paying attention to the safety mote above.

5. Continue stirring the flask until all the agarose crystals have dissolved. Allow the agarose to cool for five minutes before pouring it into the gel tray (casting).

Cast Agarose Gel

6. While the agarose is cooling, before you pour it, seal the ends of a gel-casting tray with masking tape, making sure to press the tape firmly all around the tray.

7. Carefully pour enough agarose solution into the casting tray to fill it to a depth of about 5 mm. Use a clean pipet tip to move any large bubbles out of the area where the comb will be inserted. Insert the comb. The gel should cover only about one-third the height of comb teeth. While the agarose is still liquid, a pipet tip or toothpick can be used to move large bubbles or solid debris to the sides or end of the tray.

8. The gel will become cloudy as it solidifies (This takes approximately 15 to 30 minutes depending on the percent agarose in the gel). Do not move or jostle the casting tray while agarose is solidifying.

9. When the agarose has solidified, remove the masking tape in place, gently remove comb, and remove the gel from the casting tray

10. **With gloves on**, feel the gels and compare with your classmates the relative strengths of the various w/v% gels, and then place your gel in the high-walled plastic container and cover with TBE buffer.

Load Gel

- Make certain that sample wells left by the comb are completely submerged. If you notice dimples are noticed around wells, slowly add buffer until the dimples disappear.

- Use the micropipet to add 8 µL of food coloring Sample Solution C into the wells. For each well, do the following:
    - Use a clean micropipet tip to prevent contamination
    - Steady the micropipet over well using two hands.
    - Be careful to expel any air in the micropipet tip end before loading gel. (If an air bubble forms a cap over the well, the sample will flow into the buffer around the edges of the well.)
    - Dip the micropipet tip through surface of the buffer, position it over the well, and slowly expel the mixture. Be careful not to punch the tip of micropipet through the bottom of the gel.

Student Resource 6.7

Reading: Forensic DNA

*Student Name:*_____ *Date:*_____

Directions: Use the reading below to answer the questions on the next page of this resource. If you run out of room, copy the questions and answers onto a separate sheet of paper.

Forensic DNA in the Laboratory

DNA fingerprinting is a technique that can be used to identify an individual from the DNA contained in a sample of the individual's cells. DNA fingerprinting relies on the ability of specific enzymes to cut the DNA into small pieces and the ability to analyze those pieces and compare them between samples.

DNA Fingerprinting and Polymerase Chain Reaction Forensic Techniques

One forensic technique that identifies an individual from DNA is DNA fingerprinting. DNA is found almost every cell in the body, and a blood sample contains an individual's cells. DNA fingerprinting relies on the ability of specific enzymes to cut the DNA into small pieces and the ability to analyze those pieces and compare them between samples.

DNA is cut by a type of enzyme called a restriction enzyme. Restriction enzymes occur naturally in bacterial cells as a line of defense, and their use is to cut up and deactivate foreign DNA (typically from viruses) invading the bacterial cell. The enzymes are very specific: each restriction enzyme will recognize only a certain site on a DNA molecule and cut only at that site. In the laboratory, when DNA from an individual is incubated with a specific restriction enzyme, the DNA is cut into a different number of fragments of different sizes. The number and sizes of DNA pieces will depend on the individual’s unique DNA and the specific restriction enzyme used.

After the DNA has been cut up, gel electrophoresis is used to measure the number and sizes of the DNA pieces.

DNA pieces are negatively charged. In gel electrophoresis, an electric current is applied to the DNA pieces, and they move towards the positive pole because of electrical attraction. The longer pieces move more slowly through small holes in the agarose gel and the DNA pieces separate by size. (Remember moving through your classroom matrix?) Pieces that are the same size will move to the same position in the gel.

After electrophoresis, the pieces of DNA will appear as bands in the gel. Each band contains thousands of DNA molecules, which are all the same size. This array of bands forms the pattern that is called a fingerprint. DNA pieces of known sizes (called a control) are run on the gel, and those control pieces are used to determine the size of each DNA piece of unknown size.

Human DNA fingerprinting requires one further step: in humans, the size of DNA is very large, and cutting DNA with restriction enzymes would result in an indistinguishable smear on a gel. To obtain a clear fingerprint, the DNA is transferred from the gel to a special piece of paper, called nitrocellulose, which still retains the fingerprint pattern from the gel. After this transfer, short, radioactive pieces of DNA, called probes, are incubated with the nitrocellulose. These probes will bind with specific regions of the DNA that have a complementary sequence. The sizes of these regions vary greatly among the population. When the nitrocellulose is exposed to X-ray film, the regions of DNA that bound to the probe will appear. This X-ray film is like a fingerprint in that it is unique for each person.

Another forensic technique that identifies an individual from DNA is polymerase chain reaction (PCR). This procedure also gives a fingerprint from the DNA but does not rely on using restriction enzymes. PCR

involves the use of probes to target a region of DNA, but, unlike traditional fingerprinting, these specific regions of the DNA are copied millions of times. PCR is used in the police lab when the sample from the crime scene is very small, for example a single cell of an individual. Just as in DNA fingerprinting, the DNA pieces that were copied from the sample are separated with gel electrophoresis.

Comparing DNA

Using either forensic technique, gel patterns are examined and the suspect's DNA is compared with the DNA sample found at the crime scene. The two patterns are complex, because multiple regions of DNA are probed. There is, of course, a random probability that anyone's DNA could match someone else's at one specific DNA region (each specific DNA region is called a locus). But the chance that two people's DNA will match exactly at four or five or six different regions (loci) is highly improbable. You may have heard of statistics in the range of one in a billion as the chance that two random samples could match at all loci.

If the patterns do not match exactly, then the suspect could not have contributed the DNA. In the past few years, many individuals have been released from prison based on these new techniques. If the patterns do match, the suspect is said to still be included in the sample as the probable contributor of the DNA. Combining DNA evidence with other police evidence about the suspect's motive and opportunity gives the police a better chance to catch the right criminal.

Questions

1. What are restriction enzymes?

2. What is gel electrophoresis and how does it work?

3. Why is nitrocellulose used?

4. What is a locus? How likely is it that two people's DNA would match at all loci?

Student Resource 6.8

Lab Procedure: Forensic DNA Analysis

*Student Name:*_____ *Date:*_____

Directions: In this exercise, you are going to look at the DNA evidence and analyze the results using careful forensic technique. You will analyze the forensic evidence gathered from a crime scene to create a DNA fingerprint for the victim and for each of the suspects, as well as a DNA fingerprint from the evidence DNA. It is necessary to first prepare the agarose gel in which you will analyze the DNA.

The Crime

Jayden Jones was a neighborhood success story: the kid who got a basketball scholarship to college and ended up playing in the NBA. Even though he played for a team on the other side of the country, he always came back home during the off season. So when his body was found early one morning, everyone was shocked.

He had been beaten and robbed. His hands were bruised, as if he had fought back. There was dried blood on his fingers and a trail of dried blood leading away from the body. The only other clue was an almost empty soda can lying nearby. Police took samples of the dried blood and began to look for suspects.

Suspicion immediately focused on Mark Li, who had gotten into an argument with Jones at a local nightclub the previous evening. Mark had been drinking that same type of soda during the argument. He had been a "bad kid" and was known to the police, but he hadn't been in any trouble for the last couple of years. Still, the police knew where he lived, and when they went to his apartment, they discovered a shirt in his laundry basket with bloodstains on the sleeves.

Mark insisted that he hadn't attacked Jones. He said after his argument with Jones he and his girlfriend left the club and went back to his apartment to watch TV. He claimed the blood on his shirt happened because he got a nosebleed.

Mark's girlfriend agreed with everything he said, but that didn't prove anything: she might have been trying to help him stay out of trouble. The police needed to find out if the blood on Mark's shirt was his—or Jayden Jones. They took the shirt to the police lab.

A waitress who had been working at the club that night said she heard someone arguing in the alley behind the club. She looked out the window and saw two men shouting at each other. One swung his fist and hit the other one in the face. She didn't get a good look at either of their faces but was able to describe a very unique tattoo one man had on his arm.

The police knew who had that tattoo: a convicted criminal who had recently been released from prison. They went to visit him and discovered that he looked like he'd been in a fight; he had a black eye and it looked like he might have been missing some teeth. But he insisted he was nowhere near the club on the night Jones was killed. He claimed a friend was teaching him how to box and that's how he got the black eye. Police found a blood- and sweat-stained towel in his bathroom, which he claimed was the towel he used during his boxing lessons. The police took the towel back to the police lab.

The DNA Samples

You will create a DNA fingerprint for the DNA samples that you will be testing. Use a code for each sample as follows:

- Victim: V

- Suspect 1: S1
- Suspect 2: S2
- Evidence: E

You will be looking at samples of Jayden Jones's blood and the two suspects' blood. You will be comparing those samples to the dried blood found at the scene of the crime. Your goal is to determine if the blood is Jayden Jones's or one of the two suspects'. If you confirm that it is one of the suspect's blood, you will have helped the police determine which suspect got into a fight with Jayden Jones – a fight that probably led to Jones's death.

Lab Instructions

Cast Agarose Gel

1. Seal the ends of a gel-casting tray and insert a well-forming comb. Place the tray out of the way on the lab bench so that agarose poured in the next step can solidify undisturbed.

2. Carefully pour enough agarose solution into the casting tray to fill it to a depth of about 5 mm. The gel should cover only about one-third the height of comb teeth. While the agarose is still liquid, a pipet tip or toothpick can be used to move large bubbles or solid debris to the sides or end of the tray.

3. The gel will become cloudy as it solidifies (about 20 minutes). Do not move or jostle the casting tray while the agarose is solidifying.

4. When the agarose has solidified, leave the masking tape in place and place the gel in a sealable plastic container with a small amount of TBE buffer for overnight refrigerated storage.

Transfer Gel and Load Gel

1. Remove the masking tape (or casting tray ends) and place the gel into the gel chamber so that the comb is at the negative (black) end.

2. Fill the chamber with TBE buffer to a level that just covers the entire surface of the gel.

3. Gently remove the comb, being careful not to rip wells.

4. Make certain that sample wells left by comb are completely submerged. If you notice dimples around the wells, slowly add buffer until the dimples disappear.

5. Follow the kit instructions to prepare the DNA samples.

6. Examine the wells in the gel and make sure that they are paced next to the negative electrode of the chamber. Make a drawing below to show which well will contain which sample.

7. Use the micropipet to load contents of each sample tube (Suspect 1 DNA, Suspect 2 DNA, Victim DNA, Evidence DNA) into separate wells in your gel. For each well do the following:
    - Use a clean micropipet tip for each sample to prevent contamination
    - Steady the micropipet over the well using two hands.

- Be careful to expel any air in micropipet tip end before loading gel. (If an air bubble forms a cap over the well, the sample will flow into the buffer around the edges of the well.)
- Dip the micropipet tip through the surface of the buffer, position it over the well, and slowly and continuously expel the mixture. Be careful not to punch the tip of the micropipet through the bottom of the gel.

Run Gel

1. Close the top of electrophoresis chamber and connect electrical leads to an approved power supply, anode to anode (red-red) and cathode to cathode (black-black). The anode is the positive electrode and the cathode is the negative electrode. Make sure both electrodes are connected to the same channel of the power supply.

2. Turn the power supply on and set the voltage as directed by your teacher. Shortly after the current is applied, check that the loading dye is moving through the gel towards the positive electrode, the anode.

3. Allow the DNA to migrate until the band from the loading dye nears the end of the gel.

4. Turn off the power supply, disconnect the leads from the inputs, and remove the top of electrophoresis chamber.

5. Carefully remove the casting tray and slide the gel into the staining tray labeled with your group name. Bring the gel to your teacher for staining.

Analyze Results

View the pattern of bands created by the DNA fragments.

- Does the pattern made by the DNA found at the crime scene match the pattern made by either of the suspects DNA? Based on your analysis, which DNA (if any) was found at the crime scene?

You will record your findings in your lab notebook based on your analysis of the DNA forensic evidence. Then you will work with your lab partner to finalize your lab report.

Student Resource 6.9

Peer Review: Forensic DNA Lab Report

Student Names:_____ Date:_____

Directions: Use this table to assess your partner's forensic DNA lab analysis using the rubric that follows. Give an explanation for the assessment in the last column.

Section	Assessment (circle one)	Explanation
Report Part 1: Results with Evidence and Explanation	• Exemplary • Solid • Developing • Needs attention	
Report Part 2: Possible Errors	• Exemplary • Solid • Developing • Needs attention	

Rubric: Forensic DNA Lab Report

Section	Exemplary	Solid	Developing	Needs Attention
Report Part 1: Results with Evidence and Explanation	Presents data results from experiment. Clearly describes trends and inconsistencies in the data. Proposes possible reasons for inconsistencies. Addresses the premise of the exercise: not establishing guilt or innocence but who was the source of the blood.	Presents data results from experiment. Describes trends and inconsistencies in the data. Proposes possible reasons for inconsistencies. Addresses the premise of the exercise: not establishing guilt or innocence, but who was the source of the blood.	Presents data results from experiment. Poorly describes trends and inconsistencies in the data. Does not address (or incorrectly interprets) the premise of the exercise.	Presents data results from experiment. Does not describe trends and inconsistencies in the data. Does not address (or incorrectly interprets) the premise of the exercise.
Report Part 2: Possible Errors	Identifies two or more probable sources of error. Explains how errors might have affected the results. Describes modifications to the experiment to reduce errors, and explains why the modifications would reduce error.	Identifies two or more probable sources of error. Explains how errors might have affected the results. Describes modifications to the experiment to reduce errors.	Identifies one or more probable sources of error. Explains how errors might have affected the results.	Identifies insignificant or improbable sources of error or fails to identify possible errors.

Student Resource 6.10

Assessment Criteria: Lab Notebook

Directions: Study the following criteria and ensure that your lab notebook meets them when you carry out the labs in this lesson.

List of Criteria

- Pages have numbers
- Table of contents is up to date
- Summary of experiment is included
- Writing is legible and is in black or blue ink
- Printouts are added in the right order
- Printouts are dated
- Drawings are labeled and dated
- Observations made during lab procedures are noted
- Changes to lab procedures are noted
- Mistakes in writing or drawing are crossed out with a single line and initialed
- Blank spaces are crossed out with an *X*
- Last page of experiment is signed