

Exploring DNA Extraction



HUDSONALPHA
INSTITUTE FOR BIOTECHNOLOGY

Expanded DNA Extracton

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Educational Outreach

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This kit is intended for educational purposes only. It is not to be used for research or diagnostic purposes. For additional safety information, please see the documentation included with the packing list for this activity.

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Foreword

In the broadest sense, biotechnology is the use of biological processes, organisms or systems to develop products aimed to improve some aspect of life. Biotechnology at its roots is a very old science, stretching back 7,000 years to the creation of bread, cheese, wine, and vinegar (which all depend on harnessing and modifying some biological process). The field has expanded dramatically over the last quarter century, powered by our understanding of DNA, the recipe card inside the nucleus of our cells. This recipe card provides the instructions to make proteins and all the structures of the cell.

Biotechnology combines the disciplines of molecular biology, genetics, cell biology, biochemistry, and embryology, which in turn are linked to additional fields such as chemical engineering, information technology, and robotics. Over the next few years, biotechnology is poised to heavily impact several areas of society, including healthcare, the environment and agriculture.

Career profiles of individuals who work in these fields are also included at this site:
<http://hudsonalpha.org/career-profiles>

TABLE OF CONTENTS

OVERVIEW	4
TIMEFRAME	6
LABORATORY SAFETY	6
INSTRUCTOR BACKGROUND	7
INSTRUCTOR PROTOCOL	11
EXTRACTION INQUIRY GUIDE	15
WHY EXTRACT	16
NOTES	20

Exploring DNA Extraction

Overview

This lesson will provide students with the opportunity to learn about the composition and structure of DNA. Students will be provided with a variety of plant and animal samples. They will choose which sample they would like to extract DNA from and address the specific cellular structures that serve as barriers to the extraction process. Then the students will design a lysis buffer from everyday household materials. This is done in order to break apart the cell membrane and/or the cell wall of the animal or plant cells. Students will then precipitate the DNA from their chosen material.

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Standards

2015 Alabama Course of Study 1,3

Practices

- Ask Questions
- Planning and carrying out investigations
- Analyzing and interpreting data
- Developing and using models
- Engaging in argument from evidence
- Communicating information

Cross Cutting Concepts

- Structure and function
- Cause and effect: Mechanism and explanation
- Patterns

Learning Objectives

The students will:

1. Develop an experiment taking the biological barriers and the chemistry of both DNA and the source material into account.
2. Explain the chemistry and composition of the cell wall, cell membrane and nuclear membrane and how their experimental design addresses each.
3. Build a model, or draw a diagram, or provide a text-based response using their knowledge of DNA and the chemical nature of cell structures to explain how the chemistry of their lysis solution impacted the fidelity of DNA, the structure of DNA in their product.

Materials

- coffee filters
- rubber bands
- clear plastic solo cups
- wooden dowels
- quart size storage bags & regular sandwich bags
- Ethyl alcohol OR 91% Isopropyl alcohol
- 20 ml test tube
- 50 ml test tube
- Optional: 1.5 ml tube

Various food samples:

Strawberries, kiwi, tilapia, chicken liver, dried peas soaked in warm water overnight (other foods such as bananas, tomatoes may be used. Do not use citrus fruits)

Salt Solution:

10% salt solution (protects DNA and prevents shearing and the denaturation of DNA)

Various washing agents:

Examples include: Non-ultra joy, dishwashing powder, Tide ultra, baby shampoo, Woolite and body wash

Optional Reagents

Contact solution (contains proteases-breaks down proteins), Baking soda, meat tenderizer (contains proteases-breaks down proteins),

General laboratory safety instructions can be found on page 6 of this manual.

Timeframe

There are multiple approaches that may be taken in order to plan, conduct and evaluate this lab. All components may be completed in 60 minutes.

Teachers may choose to allow students to plan their experiments a day ahead of conducting and evaluating the lab to allow more time for evaluation and discussion.

Laboratory Safety

The best protection that you have while working in the laboratory is you. Following all safety guidelines and being aware of the potential for accidents can greatly minimize the possibility of an accident occurring.

1. Always wear eye protection when working with laboratory chemicals or biological materials. The major potential for damage to the eyes due to liquids is from splashes or vapors.
2. A laboratory coat/apron should be worn at all times when working with laboratory chemicals or biological materials.
3. Gloves should be worn at all times when working with chemicals or biological materials. Remove gloves before touching commonly used surfaces such as doorknobs and computer keyboards. Wash your hands after removing gloves frequently and before leaving the lab.
4. Dispose of all materials in the appropriate disposal receptacles. When in doubt always check with the instructor.
5. Never eat or drink in the lab.
6. Always make sure you wash your hands after the lab.
7. Only closed toed shoes should be worn in the lab. Open-toed shoes or sandals are inappropriate for the lab.

Instructor Background

Exploring DNA Extraction

The purpose of this activity is to provide students with a better understanding of the chemical nature of deoxyribonucleic acid, DNA. In order to study DNA, it must be removed from living tissue. Each cell is organized to protect the integrity of its' DNA and these structures serve as physical barriers to DNA removal. The DNA is contained in the nucleus, thus both the nuclear membrane and the cytoplasmic membrane must be disrupted, dissolved, broken or damaged to release the DNA. This is the challenge facing students as they Explore DNA Extractions.

Step 1: Food Selection

Because this is an inquiry-based activity, students should have available to them a variety of food items such as strawberries, kiwis, tomatoes, fresh fish, and chicken livers from which to extract. As students consider their food choices, they should take the following into consideration.

Plant cells vs. Animal cells

One of the first challenges any scientist faces in extracting DNA is to assess the type of material from which they are extracting. Most material will fit into one of two primary categories: plant or animal. Plant cells have cell walls made mainly of cellulose fibers. If a cell wall is present, it must be broken to allow the release of the contents of the cell. Both plant cells and animal cells have cell membranes made of lipids, proteins and carbohydrates. Both plant and animal cells contain a nucleus.

Cell walls vs. Cell membranes

Cell wall composition varies depending on the species and may include proteins and polysaccharides. In plant cells, the cell wall does not serve as a major permeability barrier. It primarily serves to provide shape and strength to the cell. The major permeability barrier is the cell membrane. Note that the cell membrane is composed of a bilayer of phospholipids into which are embedded proteins and possibly carbohydrates. These molecules have hydrophilic heads and hydrophobic tails. The structure of the bilayer is maintained by hydrophobic interactions between the phospholipid tails. Any chemical that disrupts those hydrophobic forces, such as soap, will disrupt the structure of the membrane and solubilize it. The cell membrane must be disrupted to allow the release of intracellular contents.

Eukaryotic cell DNA is also encased in a second membrane, the nuclear envelope.

The nuclear envelope is composed of two lipid bilayers and protein rings or "holes" known as nuclear pores. This membrane must also be disrupted for DNA extraction.

Both plant and animal cells are organized into tissues. Tissue organization will become important later as students plan their extraction. Cell type and the chemical nature of membranes are important considerations as students plan their extraction buffer.

Step 2: Maceration

Maceration is the process of mechanically breaking apart plant and animal tissues to separate cells for maximum exposure during the extraction process. In plant tissue, this process also releases the cell contents from within the cell wall.

DNA

The code of life for living things is a molecule known as DNA, deoxyribonucleic acid. Elegant in its simplicity, it is composed of three components: deoxyribose sugar, phosphate and four nitrogen bases (adenine, thymine, cytosine and guanine). DNA has a negative electrical charge due to the phosphate groups on the DNA backbone.

The human genome consists of 3 billion base pairs of DNA, or roughly 6 feet of genetic material in every cell. For this information to fit into the nucleus, it must be substantially condensed. The basis for this condensed structure begins with the double helix or “twisted ladder” nature of this molecule resulting from its unique chemistries.

The next levels of organization result from the interaction of the negative charge of DNA with many positively charged proteins. The DNA wraps around scaffolding proteins known as histones, much like string might be wrapped around a set of beads. The wrapped histones are then coiled into larger groups called nucleosomes, which stack together to form chromatin strands. Most of the time, the chromosomes are present as long tangled chromatin strands and individual chromosomes cannot be distinguished from one another. The DNA binding proteins are also involved in opening up sections of DNA to allow transcription. Many of these binding proteins will remain with the DNA through the extraction process. In the research lab, additional steps are taken to remove associated proteins. For the purpose of this activity, associated proteins will remain intact and are present in the final extraction product. Students should consider the higher order structure of DNA as they make decisions regarding optional reagents.

Step: 3 Lysis

During lysis (derived from the word Lyse- to rupture or burst) cells are disrupted, releasing DNA from the protection of the nucleus. The lysis solutions utilized in DNA extractions typically consist of soap and salt. Other reagents may be required depending of the type of tissue from which DNA is to be extracted. The purpose of the soap in the lysis buffer is to break open the cytoplasmic and nuclear membranes by destroying them.

As seen in the HudsonAlpha lysis video clip, detergent particles, micelles, contain polar and non-polar portions that mimic the phospholipids that make up the cytoplasmic membrane. Soap molecules associate with water and the hydrophobic tails of the soap molecules associate with the hydrophobic tails of the phospholipid molecules disrupting the membrane. As membrane phospholipids are pulled into solution, non-polar tails are exposed. The exposed tails of both the membrane and micelle realign with the hydrophilic regions facing outward. The resulting micelle contains both detergent and membrane particles leaving holes in the cell/ nuclear membranes.

Soaps also help by separating the proteins found in the membranes and the cytoplasm from the DNA.

Once in the lysis solution, the positively charged ions of the salt (Na^+) are attracted to the negatively charged phosphate groups on the DNA backbone. This partially neutralizes the negative charge of DNA allowing the molecules to move more closely together or to clump. Without the positively charged sodium ions, the negatively charged DNA strands would repel, making precipitation impossible. The detergent and salt also help strip away the proteins associated with the DNA molecule.

Things to consider:

In this activity, several optional reagents are available to add to the basic lysis solution.

Contact lens solution and meat tenderizer both contain additional proteases, which will help to strip away proteins found in the membranes and cytoplasm of the cells as well as those proteins associated with the DNA molecule itself. Baking soda, otherwise known as sodium bicarbonate, is an alkaline reagent that can serve to buffer potentially acidic solutions.

Step 4: Filtration

Filtration is one process by which the bulk of the cellular debris in the lysis solution is separated from the liquid in the solution. In this activity, filtration is conducted with a coffee filter to trap the debris while allowing the liquid portion of the solution to pass through. Since DNA is soluble in water, it will be found in the liquid portion of the solution.

Student samples should be allowed to filter for approximately five minutes. During the filtration process, the teacher may wish to discuss some real world uses of DNA extraction. Alternately the teacher may choose to have students read the “Why Extract DNA?” reading passage during the filtration process.

Article found on page 16.

“Why Extract DNA?” (See article on page 16 for more detail)

Most people are familiar with forensic DNA identification and DNA paternity testing, but what else can researchers do with extracted DNA?

- Determining the exact order of nucleotide bases, DNA sequencing, allows scientists to investigate the whole genome of humans and other organisms.
- DNA sequencing of a variety of plants and plant pests points to novel methods to prevent food crop infection.
- Sequencing can be used to ensure genetic diversity of endangered species and may play a vital role in their survival.
- Cancer patients may undergo genetic testing to help doctors select the optimal treatment route.
- Patients with inherited disorders often undergo genetic tests to confirm their diagnosis.
- DNA may be used to identify pathogens much more rapidly than traditional culture methods.
- DNA evidence is currently being used to investigate ancestry and human migration patterns.
- DNA analysis of several Egyptian mummies has been used to create detailed pedigrees of pharaoh families.
- Woolly mammoth DNA from preserved arctic specimens has been analyzed for comparison with modern elephants.

Step 5: Precipitation

During precipitation, strands of DNA clump together. As more and more DNA aggregates, it forms clumps and becomes clearly visible. The texture of the precipitated DNA depends on the reagents used in the lysis solution. Isopropanol and ethanol are completely soluble in water, but are less polar than water. Because DNA is polar, it is insoluble in non-polar liquids. Thus when these alcohols are added to a water based solution of DNA, the solution become less polar and DNA falls out of solution or precipitates.

The salt in the solution plays a role in the “clumping” or aggregation of the DNA molecules during the precipitation process. The DNA becomes visible but the other cellular materials remains dissolved in the solution. DNA typically appears as stringy clumps, which have been described as ‘mucus-like’. Each glob of translucent material contains millions of clumped DNA strands.

Step 6: Spooling

Remember that DNA is a polar molecule. Because DNA is polar it dissolves in polar solutions such as water, and precipitates out in non-polar solutions like alcohol. Wood and glass are also polar, so DNA will stick to them. Students should gently twirl the wooden sticks to collect as much DNA as possible. The DNA mass can then be lifted from the alcohol solution and stored in a microcentrifuge tube. DNA stored with alcohol is highly stable and will remain intact for long periods of time. Students may notice continued clumping and condensing over time.

Anticipated Results

Results will vary. These are some general trends we have noticed in our results:

- When extracting from fish, fresh fish seems to work better than frozen and adding baking soda seems to increase the yield.
- Meat tenderizer and contact lens cleaner will often cause DNA to shear into smaller fragments that are easily visualized, but impossible to spool.
- Meat tenderizer solutions have short shelf lives. We recommend mixing this solution the day before or storing it in a refrigerator.
- Occasionally, when using baking soda as an optional reagent, spoolable DNA forms a gelatinous mass that looks very different from the anticipated cotton candy-like consistency. We suspect that this is due to sheared DNA fragments that clump together during precipitation.

Evaluate

Ask students to describe their precipitation and spooling results. Students should compare and openly discuss their results. Answers should be recorded on their inquiry guide. They should then evaluate their lysis solution in light of these observations and record the explanation on their experimental results. Students should then use their observations and their knowledge of DNA and the chemical nature of cell structures to build a model, draw a diagram or provide a text based response to explain their results. Class discussion should enrich students understanding of the relationship between DNA, chromosomes and other cellular structures. Student misconceptions concerning the role of reagents in relationship to cellular structures and the extraction process should be addressed. Ask students what new research questions they may have to help them further understand the relationships between DNA, chromosomes and other cellular structures.

Instructor Protocol

Prior to Day One

1. Make copies of the Expanded inquiry guide for each pair of students in the class. The guide is found on page 15 of this manual. *Permission is hereby granted to teachers to reprint or photocopy in classroom quantities the pages or sheets needed for the students.*
2. Dispense 3 ml of washing agents into test tubes. Label test tubes or racks with a description of each agent *If you use powdered dishwashing agent make a 10% solution (10g of powder and bring the volume to 100 ml with water) and dispense. *OPTIONAL: If you want to give the students practice measuring, you can simply place the containers of washing agents on the lab bench and provide 10 ml graduated cylinders for each solution.*
3. Dispense 2 ml of each of the optional reagents. Label test tubes or racks with a description of each reagent. *OPTIONAL: If you want to give the students practice measuring, you can simply place the containers of optional reagents on the lab bench and provide 10 ml graduated cylinders for each solution.*
4. Prepare food samples: quarter the kiwi, cut the tilapia/chicken livers into ~ 4 cm X 4 cm pieces, and soak the dried peas in warm water overnight. Place foods in their respective weigh boats. *Note: Place cut tilapia/chicken liver in sandwich bags.*
5. Dispense 25 ml of ethyl/isopropyl alcohol into 50ml test tubes. Place in freezer. *If a freezer is not available, place tubes in a cooler with ice overnight.*
6. Optional – Dispense 100 ul of ethyl/isopropyl alcohol into 1.5 ml microcentrifuge tubes
7. Optional – Prepare an extraction kit/student. Take a quart size sandwich bag and place within each bag a: sandwich bag, cup, coffee filter, rubber band, 1.5 ml microcentrifuge tube, and a wooden dowel

Day One

Content & Media Connections	Procedure	
Discuss the differences in plant and animal cells <i>Media Connection:</i> HudsonAlpha iCell	Have students select their food sample and record their choice on the inquiry guide. Students should write a statement explaining how they think this would affect the extraction process and describe cellular structures that serve as barriers.	Planning
	Students should get their food sample and place it in the sandwich baggie. Note: have the students get as much air as possible out of the bag before sealing.	
Review chemical composition of cell walls and cell membranes. <i>Media Connection:</i> HudsonAlpha maceration video clip HudsonAlpha iCell	Maceration - Students smash their food sample in the baggie. Note: this works best by pressing with thumb and pointer fingers, not pounding.	
Discuss cell membrane composition and polarity of phospholipids. Describe the action of soap in relation to lipid composition of the membrane. <i>Media Connection:</i> HudsonAlpha lysis action video clip	Designing the lysis solution- Introduce students to the available soaps from which to choose to make their lysis buffer. Have the students record their selection on the Inquiry guide with an explanation for how each reagent will aid in the extraction process.	
Review overall negative charge of DNA molecules.	Explain that salt is a key component to every lysis solution. The salt plays a role in maintaining the structural stability of the DNA.	
Relate the role of proteins, such as histones, to maintaining the higher order structure of DNA.	Introduce students to the additional reagents that may be added. Discuss the overall use of each reagent. Students may choose 1, 2 or none of the reagents. If students choose to add a reagent, they must explain why they chose to add it in the space provided on the inquiry guide.	

Content & Media Connections	Procedure	
Encourage students to record volumes of each solution before they mix the various agents in a single tube.	<p>Students will make their own buffer solution by:</p> <p>Choosing 1 soap solution</p> <p>Taking 1 salt solution</p> <p>Choose one, two or none of the optional reagents.</p>	Experimentation
	<p>Bring solution to volume. Have students add water to bring the total lysis buffer volume to 15 ml. Students should gently invert their solutions to ensure mixing.</p> <p>* Students will briefly outline the procedure on the guide.</p>	
	<p>Add the entire lysis buffer to the macerated food sample in the baggie. Mix food sample and lysis buffer together ~ one minute, by palpating the outside of the bag.</p>	
Define filtration. Ask students to elaborate on the role of filtration in DNA extraction.	<p>Filtration - Take a coffee filter and place it in a solo cup so that the edges of the filter surround the outside of the cup and there is an indentation in the middle. Secure the filter with a rubber band.</p>	
Discuss DNA extraction applications, or have students read the “Why Extract?” article during the filtration process.	<p>Pour the food sample/lysis buffer mixture into the filter and allow the filtrate to flow through the filtration device. (Approx. 5min.)</p>	
Ask where students would expect to find dissolved DNA in the liquid filtrate: at the bottom of cup or the solid matter in the filter paper ?	<p>Remove filter by holding the top of the filter and sliding the rubber band down the cup. Lift the filter and its contents. Place the filter into the quart plastic baggie, “trash”. Save filtrate for the next step.</p>	
Define precipitation. Review DNA solubility in water and alternately alcohol.	<p>Precipitation - Pour filtrate into alcohol solution.</p>	

Content & Media Connections	Procedure	
	Instruct students to carefully observe the interface of the two solutions and record their observations in the appropriate space on the inquiry guide	
Define spooling. Remind students that not all groups' DNA will be intact enough to spool.	Spool DNA with wooden dowel. Twirl the stick and spin up the DNA, like you would cotton candy. Note: not all students may be able to spool their DNA. Results should be recorded on the inquiry guide.	
Caution students to keep DNA in the tube once they leave the lab. Ask students to estimate the 'shelf-life' of alcohol stored DNA.	Students may take their DNA and place it in the 1.5 ml tube filled with 100 ul of ethyl/isopropyl alcohol * With proper storage, students' DNA samples will remain intact for years.	
Here students should make the connections between the lysis solution they created, how the chemistry of the solution impacted the fidelity of DNA, and structure of DNA.	Students should compare their results paying attention to the consistency, quality and quantity of DNA extracted and openly discuss their results. They should then evaluate their lysis solution in light of these observations and record the explanation of their experimental results. .	Communicate
Student misconceptions concerning the role of reagents in relationship to cellular structures and the extraction process should be addressed	Students should then use their observations and their knowledge of DNA and the chemical nature of cell structures to build a model, draw a diagram or provide a text based response to explain their results. Class discussion should enrich students understanding of the relationship between DNA, chromosomes and other cellular structures.	
	Ask students what new research questions they may have to help them further understand the relationships between DNA, chromosomes and other cellular structures.	

Expanded DNA Extraction

Name _____

DNA Source	Answer the following questions about your DNA source
	Describe the features of your source that are likely to impact the extraction process. Ex: high acidity or hard covering
	Describe the cell structures that serve as barriers to extracting intact DNA:

Lysis Solution Components			
Component	Name	Volume Added	Provide a scientific explanation of what you think these reagents will do to aid in your extraction
Washing Agent			
Salt Solution	NaCl	2 mL	
Additional Reagent (Optional)			
Additional Reagent (Optional)			
Additional Water	H ₂ O		If needed, to bring total volume to 15 mL. Total volume of ALL reagents cannot exceed 15 mL.

Basic DNA Extraction Steps	Briefly outline the procedure you will use to extract intact DNA.
1. Maceration 2. Lysis 3. Filtration 4. Precipitation 5. Spooling	

Describe the DNA precipitation and spooling results	
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Expanded DNA Extraction

Name _____

Compare your results to other groups. You may notice differences in the amount, consistency or quality of extracted DNA.

What part of your procedure or lysis solution do you think is primarily responsible for your extraction results? Why?	
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Use your knowledge of DNA and the chemical nature of cell structures to explain your results. You may build a model, draw a diagram or provide a text-based response. Your response should include information about your unique lysis solution.

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One goal of this activity is to understand relationships between DNA, chromosomes and other cellular structures. You may have answered some of these questions by doing this experiment, but what new questions do you have? What other research questions would help explain or illustrate those relationships?

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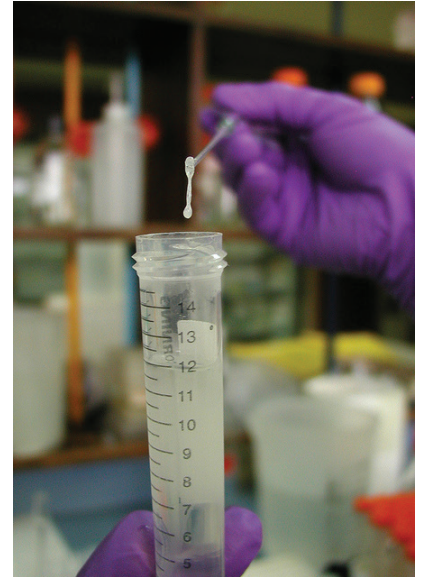
Expanded DNA Extraction

Name _____

Why Extract?

The Crime Scene investigator holds up the brightly colored swab and says, “We’ve got DNA.” A few scenes later, the criminal has been found and handcuffed. This is a common theme on many television crime shows. DNA evidence is often presented in courtrooms as well as television dramas. Most people are familiar with forensic DNA identification and DNA testing for paternity, but what else can researchers do with extracted DNA?

Isolating DNA from living, or once living tissue is an important first step in many lab processes. Doctors may send patient samples for a wide variety of DNA-based tests. Cancer patients may have DNA testing to help doctors select the most promising treatment routes. Patients with suspected inherited disorders often have genetic tests performed to confirm their diagnosis. DNA isolated from tissues may also be used to diagnose infectious disease. When a sick person visits the doctor’s office, a sample of blood, urine, saliva or mucus may be collected. This sample is then cultured and allowed to grow. The technician examines the cultures with a microscope to identify the type of bacteria or virus that is making the patient sick. This may take several days or even weeks. New techniques allow for much faster results by isolating DNA from the sample and comparing that DNA to known disease causing agents. If the DNA from the sample matches the DNA from the bacteria that causes pneumonia then the patient has bacterial pneumonia. Quick identification of the type of disease agent means that doctors can treat patients more effectively.



Recently DNA has been extracted from fossilized remains. In 2008, DNA was isolated from an extinct species, the woolly mammoth. Researchers were able to use preserved hair samples of more than 10 mammoths to extract high quality DNA samples. Following sequencing, mammoth DNA was compared to the DNA of modern elephants. Through these studies, researchers hope to learn more about the reasons for mammoth extinction and more about the biological history of modern elephants. DNA extracted from bone fragments of three female Neanderthal skeletons were used to produce a near complete Neanderthal genome in 2009. Scientists were able to extract fragmented DNA from skeletal remains, sequence the 30 to 50 million base pair fragments and align those short sequences to produce the 3 billion base pair sequence. Comparisons of Neanderthal DNA to sequences of modern humans revealed surprising evidence that early humans and Neanderthal populations mingled and interbred. Extracted DNA from Egyptian mummies has been used to create a family tree for King Tut. Detailed analysis of mummy DNA has revealed a fascinating portrait of royal lineages in prehistoric Egypt.

DNA Sequencing is finding the order of the letters that make up the language of the genome. While the term gene refers to specific piece of DNA that provides instructions to the cell, genome refers to the entire DNA in an organism. The Human Genome Project used scientists from across the world to build a reference sequence for all human chromosomes. Since the projects’ completion in 2000, researchers have used that reference sequence to find causes of rare genetic disorders, link specific genes to certain diseases and find variations among different peoples. Scientists have now begun the task of determining how different parts of the genetic code work together.



In addition to humans, the genomes of many other organisms have been sequenced. Knowing the genome of an organism can help identify interesting and useful genes or help scientists understand how species change over time. Researchers may sequence the genome of a fungus that causes diseases in plants in the hopes of finding ways to prevent the fungus from infecting crops. Plants such as sorghum and algae have been sequenced to learn how these species might be used as sources of biofuels. Endangered species, such as Tasmanian devils and tigers have been sequenced to help in efforts to save them from extinction.

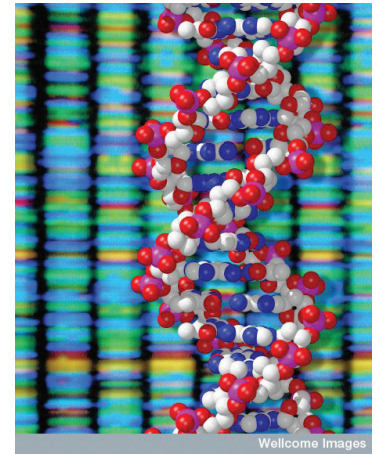


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NOTES

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