
Biotechnology Explorer™

Genes in a Bottle Kit DNA Extraction Module

**Catalog Number
166-2000EDU**

**DNA necklace module (166-2200EDU)
must be purchased separately.**

explorer.bio-rad.com

See individual components for storage temperature.

Duplication of any part of this document is permitted for classroom use only.

BIO-RAD

Capture Your Essence!

Whether it's being cloned, sequenced, fingerprinted, mapped, or genetically engineered, DNA has become an everyday topic in the media and the classroom. Introduce your students to the molecular framework of biology — with their own essence! Your students can capture, preserve, and even bottle their own DNA.

How do scientists separate pure DNA from cells composed mainly of lipids, proteins, carbohydrates, and salts? Membranes are first ruptured with detergents to release DNA into a solution; then proteins and other organic molecules are digested and separated while the DNA remains intact. The DNA is finally collected by precipitation in a form that can be manipulated as desired.

With this simple laboratory activity, students gain practical knowledge by conducting a real-world laboratory procedure that is used to extract DNA from many different organisms for a variety of applications. Your students will extract genomic DNA from their own cheek cells and watch it precipitate from solution as floating white strands. Using the DNA extraction module (166-2200EDU), the DNA strands are then easily collected and transferred to a glass vial, and the vial is fashioned into a necklace.

This kit is suitable for students from 5th grade through college and minimal background knowledge is required. This laboratory activity can be performed at any point during a typical biology or life science course, when topics such as cells, cell structure, mitosis and meiosis, genetics, and DNA technology are discussed.

For students learning about the molecular framework of biology for the first time, DNA is abstract and intangible. This procedure makes the invisible visible — seeing their own DNA makes it real. Illustrations of DNA and additional laboratory activities develop students' understanding of DNA's function as the genetic blueprint, and help students comprehend this previously invisible substance of life.

This kit provides learning opportunities for all levels of instruction. The activity is designed for any classroom environment and requires no specialized equipment or stains. For secondary and college level instruction, lessons on DNA structure and function, cell structure, and enzyme function can be introduced or reinforced with this laboratory activity. For middle school students, it's a perfect introduction to the exciting world of DNA science.

We welcome your comments and suggestions. Have fun!

Nebbie Idris, PhD
Biotechnology Explorer
Product Manager

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Teacher's Guide

DNA Extraction Module Inventory and Supplies

The materials in this kit are sufficient for 36 students.

Kit Contents	Amount Provided
Lysis buffer	40 ml
Protease	1.3 ml
5 M sodium chloride (salt)	5 ml
Sterile water	2.5 ml
5 ml round-bottom test tubes	50
Clear micro test tubes	60
Multicolor micro test tubes	60
Clear, capless screwcap tubes	40
Assorted color screwcaps	40
Disposable plastic transfer pipets	50
Foam micro test tube holders	10
Cytology brushes	80
Parafilm	1 strip

Required Accessories (not included in this kit)	Amount Required
91% isopropanol (available at drug stores) or 95% ethanol	approx. 250 ml
Water bath with thermometer, set at 50°C*	1
Permanent markers	1–9
Container of ice	1
Disposable paper cup or beaker for waste disposal	9

Optional DNA Necklace Module (not included in this kit)**

166-2200EDU contains:

Glass vials	18
Silver caps	18
Plastic plugs	18
Waxed string	18
Super glue gel	1 tube

* If a temperature-controlled water bath is not available, use one or more insulated containers (Styrofoam is best) large enough to hold the foam micro test tube holders, and fill with water heated to 50°C.

**Each DNA necklace module contains enough material to prepare 18 necklaces. Two kits are required for a class of 36 students.

Cheek Cell DNA Extraction

Capture Your Genetic Essence in a Bottle

Overview for the Teacher

Why Should You Teach DNA Extraction?

- 1) **DNA extraction gives students the opportunity to see their very own genetic essence.**

You and your students will be excited to see the very substance that makes them unique become visible before their eyes. The precipitated DNA can be sealed and stored in an attractive glass vial that can be treasured for a long time.

- 2) **DNA extraction helps students to understand properties of DNA.**

The DNA molecules that make up our chromosomes are incredibly long and thin. Ask your students to imagine how such long molecules can fit into microscopic cheek cells. The fine white fibers that they will see as their DNA precipitates is many thousands of DNA molecules wound over each other like fibers in yarn.

- 3) **DNA extraction is the first step in DNA technology.**

DNA extraction is a routine step in many biotechnology procedures: Gene cloning, gene mapping, DNA sequencing, and DNA fingerprinting all require that DNA be extracted and isolated from their cell or tissue sources. With this activity, students can get an idea of how easily DNA can be isolated for use in cutting-edge research.

Intended Audience

This laboratory is appropriate for students from 5th grade through college, as a first introduction to DNA or as a quick, easy, and impressive hands-on accompaniment to existing DNA instruction. Even students who have previously extracted DNA out of onions or liver will find extracting their own DNA far more relevant and exciting.

The instruction manual includes content for both advanced instruction (9th grade through college) and basic instruction (5th through 8th grades). Depending on the needs of your students, you may choose to include activities or background material from either section. **A complete student manual is provided for both levels of instruction.**

Curriculum Fit

This laboratory activity can be performed at any point during a typical biology or life science year, but it is particularly relevant when the following topics are being discussed:

- Biomolecules
- Cell structure
- Mitosis and meiosis
- Genetics
- DNA technology

Recommended Student Background

High school students should have a general appreciation for the structure and function of DNA before starting this activity. No prior knowledge of DNA structure or function is expected for middle school students.

Activity Timeline

This laboratory activity can be performed easily in one 50-minute class period but can be expanded to include several extension activities.

Lesson 1	Introduction and background material
Lesson 2	Cheek cell isolation, DNA extraction, and precipitation
Lesson 3	DNA necklace preparation (optional)

Safety Issues

Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Wearing protective eyewear and gloves is strongly recommended. Students should wash their hands with soap before and after this exercise. If any of the solutions gets into a student's eyes, flush with water for 15 minutes.

Keys to Success

Ample cell collection is critical for success. For best results, make sure students spend the recommended amount of time collecting and carefully transferring cheek cells.

Volume Measurements

This kit was developed for use in classrooms with minimal laboratory equipment and limited knowledge of scientific techniques. Micropipets are not required but can be used to transfer liquids.

Background and Fundamentals for Basic Level Instruction

What is DNA and what does it do?

Deoxyribonucleic acid (DNA) is a molecule present in all living things, including bacteria, plants, and animals. DNA carries genetic information that is inherited, or passed down from parents to offspring. It is sometimes referred to as a biological “blueprint” because it determines all of an individual’s physical features such as hair, eye, and skin color, height, shape of facial features, blood type, and countless others. Your DNA blueprint is a combination of your mother’s DNA (from her egg) and your father’s DNA (from his sperm) during conception.

DNA contains four chemical units, referred to by the first letters in their names: **A** (adenine), **G** (guanine), **T** (thymine), and **C** (cytosine). These four letters make up a code for genetic information. The letters of the DNA code function like letters of our alphabet. The 26 letters in the English alphabet spell words, which can be arranged in infinite ways to create messages and information. Similarly, the 4 chemical letters of DNA are organized to make messages that can be understood by cells, called **genes**. These genes contain the information to make **proteins**, which are the basis for almost all of a body’s and cell’s structures and functions.

Your DNA sequence is the particular arrangement or order of the chemical letters within your complete DNA collection, or **genome**. Scientists have determined that human DNA sequences are 99.9% identical. It is the <0.1% sequence variation from person to person that makes each of us unique.

Where is DNA found?

With only a few exceptions, DNA is found within practically every cell of an organism’s body. In our cells, a compartment of the cell called the **nucleus** contains the DNA. Every time a cell divides (for growth, repair, or reproduction) the DNA within the cell’s nucleus is copied and then coiled tightly into **chromosomes**. The human genetic blueprint is organized into 46 chromosomes, which contain approximately 40,000 genes that provide the instructions for constructing the human body.

What does DNA look like?

At the molecular level, DNA looks like a twisted ladder or a spiral staircase. The ladder actually contains two strands of DNA, with pairs of the chemical letters **A**, **G**, **T**, and **C** forming the rungs. This structure is called a DNA **double helix** because of the spiral, or helical form made by the two DNA strands. Each strand of DNA is very long and thin and is coiled very tightly to make it fit into the cell's nucleus. If all 46 chromosomes from a human cell were uncoiled and placed end to end, the DNA would be 2 meters long — but only 2 nanometers (2 billionths of a meter) wide.

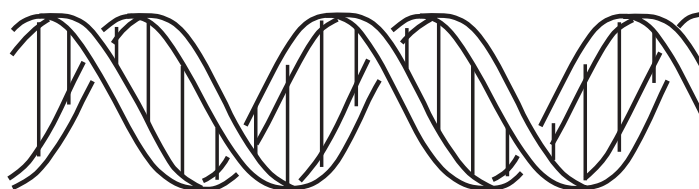


Fig. 2. A schematic representation of DNA (deoxyribonucleic acid). DNA is a long chainlike molecule that stores genetic information.

How can we make DNA visible?

We can see our DNA by collecting cells, breaking them open, and condensing the DNA from all of the cells together. Think of the long, thin DNA molecules as thin white threads. If the threads were stretched across a room they would be difficult to see, but piled all together on the floor they would be visible. This laboratory activity uses detergent and enzymes to break open cells collected from students' cheeks and release the DNA from within them. Salt and cold alcohol are then added to make the DNA come out of solution, or **precipitate**, into a mass that is big enough to see.

Background and Fundamentals for Advanced Level Instruction

Applications of DNA Technology

This laboratory activity can be integrated into classes that discuss DNA structure and function and can be used to give students a simple, hands-on experience with their own DNA. It takes on even more significance if students understand that DNA extraction is the first step of many biotechnology applications, such as:

Cloning

Cloning means to make many copies of a fragment of DNA or genome. A defective gene that causes disease may be cloned so that it can be sequenced and analyzed toward the goal of finding a cure. A gene encoding a desirable protein or trait may be cloned so that it can be inserted into another organism (see Gene Transfer below). Likewise, an entire genome can be cloned by inserting it into cell nuclei that are capable of developing into organisms.

Gene Transfer: Genetically Modified Organisms (GMOs)

To produce useful quantities of a valuable protein, such as a human blood clotting protein, the gene that codes for the protein is isolated and moved into cells that can be grown quickly and in quantity. These cell “factories” can be bacteria, yeast, mold, plants, or animal cells.

Sometimes a mammal is used to produce the desired protein. A gene that codes for a desirable protein may be inserted into a fertilized cow egg. The genetically modified cow will produce the desired protein in its milk, from which the desirable protein can be extracted.

Agricultural crops now contain genes from other organisms. For example, some plants contain a gene that codes for a protein that kills caterpillars. Other plants contain genes that enable them to withstand herbicides so that farmers can spray a whole field with herbicide, killing all the weeds and allowing the crop to survive.

DNA Profiling

Using a technique called the polymerase chain reaction (PCR), scientists can study specific regions of chromosomes where individuals’ DNA sequences differ, and amplify, or make many copies of them (creating sufficient quantities of these sequences to manipulate and analyze). Using gel electrophoresis, the differences between individuals can be displayed as banding patterns that resemble bar codes. This technique can be used to solve crimes, test paternity, and also to determine the evolutionary relatedness of organisms.

Extraction and Precipitation of DNA: How Does It Work?

Students will start this activity by scraping cells from the inside of their mouth and placing the collected cells into a tube containing lysis buffer. The lysis buffer contains a detergent that breaks apart the phospholipid cell membrane and nuclear membranes, allowing the DNA to be released. It also contains a buffering agent to maintain the pH of the solution so that the DNA stays stable.

Protease, an enzyme that digests proteins, is added to remove proteins bound to the DNA and to destroy cellular enzymes that would digest the DNA. This insures that you maximize the amount of intact DNA that is extracted. The cell extract containing protease is incubated at 50°C, the optimum temperature for protease activity.

DNA and other cellular components, such as fats, sugars, and proteins, dissolve in the lysis buffer. DNA has a negative electrical charge due to the phosphate groups on the DNA backbone, and the electrical charge makes it soluble. When salt is added to the sample, the positively charged sodium ions of the salt are attracted to the negative charges of the DNA, neutralizing the electrical charge of the DNA. This allows the DNA molecules to come together instead of repelling each other. The addition of the cold alcohol precipitates the DNA since it is insoluble in high salt and alcohol. The DNA precipitate starts to form visibly as fine white strands at the alcohol layer boundary, while the other cellular substances remain in solution.

Teacher's Laboratory Guide

This section presents an overview and lesson flow, advance preparation, student workstation setup, and techniques and concepts to highlight.

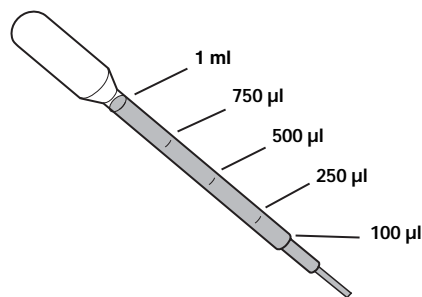
Implementation Timeline

1–2 days	Lesson 1	Introduction and background material Optional dry laboratory demonstration of DNA extraction — recommended for students in grades 5–8. See extension activities at the end of the manual.
50 minutes	Lesson 2	Cheek cell isolation, DNA extraction, and precipitation
30–50 minutes	Lesson 3	Optional DNA necklace preparation

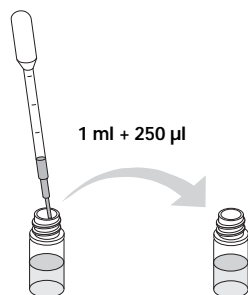
Teacher's Advance (Pre-laboratory) Preparation For Lesson 2

- **Volume Measurement**

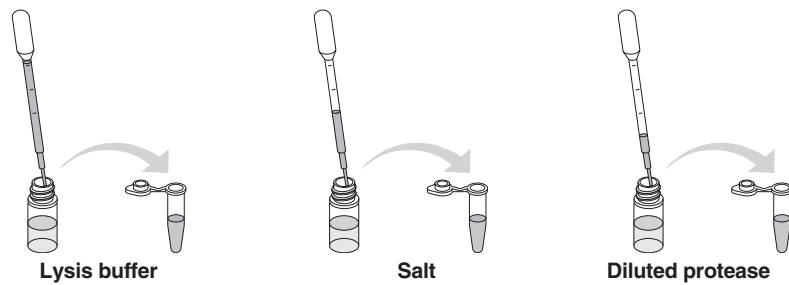
This kit contains graduated disposable plastic transfer pipets that will be used for all the liquid measurements. The diagram below shows marks on the pipet corresponding to the volumes you will be measuring. Digital micropipets may also be used.



- Place the alcohol (isopropanol or ethanol) in the freezer at least 1 hour before beginning this laboratory.
- Add 1.25 ml of water (1 ml + 250 µl) to the bottle containing protease to dilute the protease. Gently invert the bottle 5 times to mix the water and the protease. Once diluted, the protease solution may be stored at 4°C for up to 2 months.



- Aliquot the lysis buffer, diluted protease, and sodium chloride (salt) solutions into the appropriate flip-top micro test tubes. See detailed instructions below.



- Cut the sheet of Parafilm into 36 or more small squares, to provide one square per student.

Aliquotting of Solutions for Each Student Workstation (4 students/station)

1. For each student, aliquot 1 ml of **lysis buffer** in a clear micro test tube (up to 4 tubes per station).
2. Aliquot 500 μ l (0.5 ml) of sodium chloride (salt) into 8 pink micro test tubes and label the tubes "**salt**".
3. Aliquot 250 μ l of the diluted protease (see p. 8 for dilution instructions) into 8 blue micro test tubes and label the tubes "**prot**".
4. Place 4 clear micro test tubes of **lysis buffer**, 1 pink micro test tube labeled "**salt**", and 1 blue micro test tube labeled "**prot**" in a foam micro test tube holder to place at each student workstation.

DNA Extraction and Precipitation

Workstation Checklist

The materials in this kit are sufficient for 36 students.

Teacher's (Common) Station

Water bath at 50°C

Ice-cold bottle of 91% isopropanol or 95% ethanol on ice

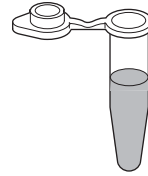
Students' Workstation (4 students per station)	Number required
Clear micro test tubes, each containing 1 ml lysis buffer	4
Blue micro test tube labeled "prot", containing 250 µl of protease	1
Pink micro test tube labeled "salt", containing 500 µl of salt	1
Clear, capless screw cap tubes	4
Assorted colored screw caps	4
Cytology brushes	8
5 ml round-bottom test tubes	4
Parafilm (small pre-cut pieces)	4
Disposable plastic transfer pipets	4
Foam micro test tube holder	1
Permanent marker	1
Disposable paper cup or beaker for waste collection	1

Notes to the instructor

Ample cell collection is critical for success. For best results, make sure students spend the recommended amount of time collecting and carefully transferring the cells.

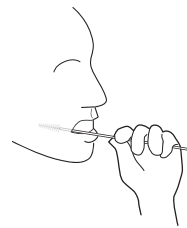
Quick Guide for DNA Extraction and Precipitation

1. Obtain for yourself a clear micro test tube containing 1 ml of lysis buffer from the foam micro tube holder at your workstation, and label it with your initials using a permanent marker.

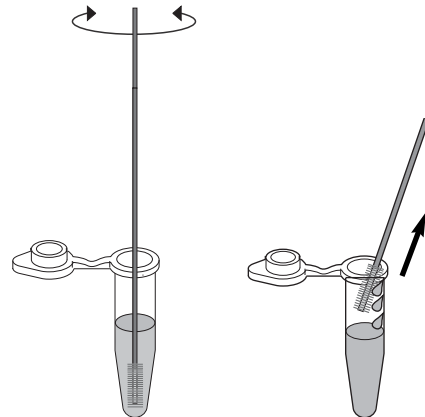


1 ml lysis buffer

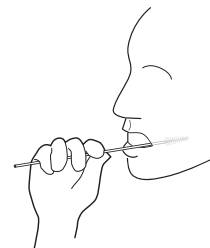
2. Gently scrape cells from the inside of your right cheek and from the space between your cheek and gum with a brush for 1 minute; try to collect as much cell material as possible.



3. Place the brush with the cheek cells into the tube containing lysis buffer. Swirl the brush around to release the cells from the brush into the buffer. Scrape the brush bristles across the top of the tube to transfer as much of the cells into the micro test tube as possible.

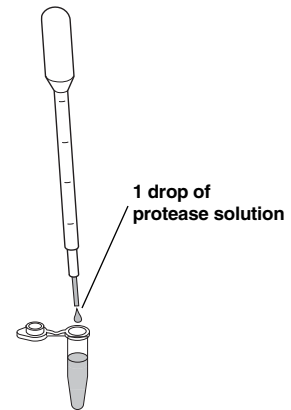


4. Using a second, clean brush, gently scrape the cells from the inside of your left cheek, in between your cheek and gum, along the roof of your mouth, and under your tongue for 1 minute; again, try to collect as much cell material as possible. Place the brush in and transfer the cells to the same tube as before.

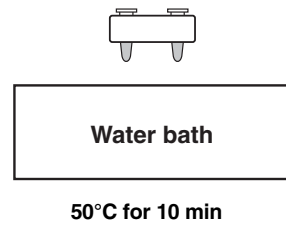


5. Cap the micro test tube and **gently** invert it 5 times to mix.

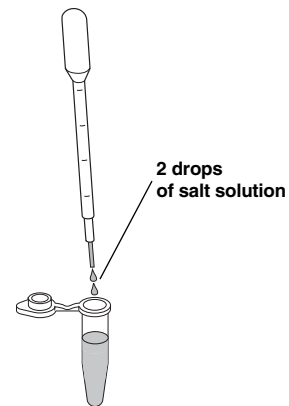
6. Using a plastic transfer pipet, add 1 drop from the tube labeled "**prot**" into the tube containing your cells. Cap the cell extract tube and invert it 5 times to mix.



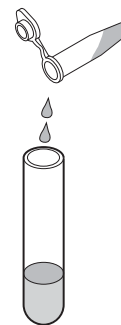
7. Place your group's micro test tubes in the foam micro test tube holder and incubate them at 50°C for 10 minutes. Remove your tubes from the water bath.



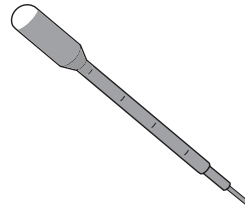
8. Using a plastic transfer pipet, add 2 drops from the tube labeled "**salt**" into the tube containing your cell extract. Cap the tube and gently invert 5 times to mix.



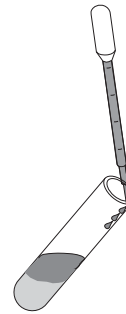
9. Label a clean 5 ml round-bottom test tube with your initials and pour the contents of your micro test tube into the round-bottom tube.



10. Obtain a plastic transfer pipet and fill it with cold alcohol.



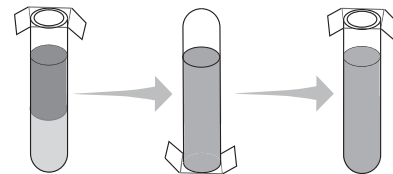
11. Tilt the round-bottom tube at a 45° angle and slowly add the alcohol, carefully letting it flow gently down the inside wall of the tube.



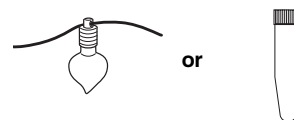
12. Let the tube sit upright and undisturbed for 5 minutes.



13. After 5 minutes, seal the top of the tube with a piece of Parafilm and slowly invert the tube 5 times to help the DNA, which has begun to precipitate, to aggregate.



14. With a plastic transfer pipet, carefully transfer the precipitated DNA along with approximately 750 μ l to 1 ml of the alcohol solution into a small glass vial provided in the DNA necklace kit (166-2200EDU), or, if you are not going to make a DNA necklace, save your DNA in a screwcap tube provided in this kit.



Student Manual: Basic Instruction

Cheek Cell DNA Extraction Capture Your Genetic Essence in a Bottle

Contents

- Lesson 1** Introduction and background material, dry laboratory extension (optional)
- Lesson 2** Cheek cell isolation, DNA extraction, and precipitation
- Lesson 3** DNA necklace preparation (optional)

Student Manual: Basic Instruction

Cheek Cell DNA Extraction Capture Your Genetic Essence in a Bottle

Introduction

What is DNA and what does it do?

Deoxyribonucleic acid (DNA) is a molecule present in all living things, including bacteria, plants, and animals. DNA carries genetic information that is inherited, or passed down from parents to offspring. It is responsible for determining a person's hair, eye, and skin color, facial features, complexion, height, blood type, and just about everything else that makes an individual unique. But it also contains all the information about your body that is the same in all human beings. In other words, your DNA is like a blueprint for your entire physical growth and development. Your DNA blueprint is a combination of half of your mother's and half of your father's DNA, which is why you have some features from each of your parents.

DNA contains four chemical units, referred to by the first letters in their names: **A** (adenine), **G** (guanine), **T** (thymine), and **C** (cytosine). These four DNA "letters" make up a code for genetic information. The letters of the DNA code are similar to the letters of our alphabet. The 26 letters in our English alphabet spell words, which can be arranged in infinite ways to create messages and information. Similarly, the 4 chemical letters of DNA are organized to make messages, called **genes**, that can be understood by cells. These genes contain the information to make **proteins**, which are responsible for almost all of your body's structures and functions. A gene is like a recipe, since it contains the all the information needed to make a protein.

Your DNA sequence is the particular arrangement or order of the chemical letters within your complete DNA collection, or **genome**. Scientists have determined that human DNA sequences are 99.9% identical. It is the <0.1% sequence variation from person to person that makes each of us unique. In other words, what makes you different from your classmate is an occasional difference in the letters of your genomes.

Where is DNA found?

The basic units of an organism's body are cells — they make up all of your tissues and organs (e.g., muscles, brain, digestive system, skin, glands, etc.) Cells are compartments with membranes, made of protein and lipids (fats), that keep them separate from other cells. Within cells are further compartments with specialized functions. One compartment, called the **nucleus**, is like the cell's control headquarters and contains the DNA molecules, which are the master instructions for the functions of the cell. The DNA is organized into 46 tightly coiled structures called chromosomes. Every time a cell divides to make two identical new cells — for growth, repair, or reproduction — the chromosomes are copied, ensuring that the new cells will receive a full copy of the genetic blueprint for the organism.

What does DNA look like?

At the molecular level, DNA looks like a twisted ladder or a spiral staircase. The ladder actually contains two strands of DNA, with pairs of the chemical letters **A**, **G**, **T**, and **C** forming the rungs. This structure is called a DNA **double helix** because of the spiral, or helical form made by the two DNA strands. Each strand of DNA is very long and thin and is coiled very tightly to make it fit into the cell's nucleus. If all 46 human chromosomes from a cell were uncoiled and placed end to end, they would make a string of DNA that is 2 meters long and only 2 nanometers (2 billionths of a meter) wide!

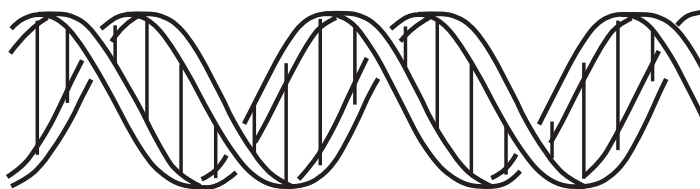


Fig. 2. A schematic representation of DNA (deoxyribonucleic acid). DNA is a long chainlike molecule that stores genetic information.

How can we make DNA visible?

Step 1: Collect cells

To see your DNA, you will collect cells, break them open, and condense the DNA from all of the cells together. You can collect thousands of cells from the inside of your mouth just by scraping it gently and thoroughly with a brush. The type of cells that line your mouth divides very often, coming off easily as new cells replace them continuously. In fact, these cells are coming off and being replaced every time you chew and eat food.

Focus question:

1. How could you test whether you were actually collecting cells from your cheeks? What piece of laboratory equipment might you use?

Step 2: Break open (lyse) the cells

Once you have collected your cells, the cells need to be broken open to release the DNA. Detergent will dissolve the membranes of your cells, just like dishwashing detergent dissolves fats and proteins from a greasy pan, because cell and nuclear membranes are composed of fats and proteins. Dissolving the membranes results in the release of the DNA. The process of breaking open the cells is called **lysis**, and the solution containing the detergent is called **lysis buffer**.

Focus questions:

2. When washing dishes, what works better, warm or cold water? Which do you think will help the detergent break open the cell, warm or cold temperatures?

3. Do you think your DNA will be visible after you have broken open your cells? Why or why not?

Step 3: Remove proteins

DNA is packaged tightly around proteins. Like spools for thread, these proteins keep the DNA tightly wound and organized so that it doesn't get tangled inside the nucleus. For you to see the DNA, it helps to remove the proteins so that the DNA can first loosen and expand, then collect into a mass with the DNA from all the other cells. You will incubate your lysed cheek cells with **protease**, which breaks down proteins so that they can no longer bind DNA. Protease is an **enzyme**, or protein machine, that works best at 50°C, which is the temperature of slightly hot water. The protease chews up the proteins associated with the DNA and also helps digest any remaining cell or nuclear membrane proteins.

Focus question:

4. Where do you think you would find proteases in your body? **Hint:** Where do the proteins that you eat get broken down?

Steps 4 and 5: Condense the DNA

Strands of DNA are so thin that it is not possible to see them when they are dissolved in solution. Think of the long, thin strands of DNA as fine white thread. If one long piece of thread were stretched across the room, it would be difficult to see. To make the thread more visible, you could collect it all together and pile it on the floor. In this laboratory experiment, you will use salt and cold alcohol to bring the DNA out of solution, or **precipitate** it. Salt and cold alcohol create a condition in which DNA doesn't stay in solution, so the DNA clumps together and becomes a solid mass that you can see.

Focus question:

5. Have you ever tried to add sugar to iced tea? Does the sugar dissolve easily? How does this compare to dissolving the same amount of sugar in the same amount of hot tea?

What does precipitated DNA look like?

Like salt or sugar, DNA is colorless when it is dissolved in liquid, but is white when it precipitates in enough quantity to see. As it precipitates, it appears as very fine white strands suspended in liquid. The strands are somewhat fragile — like very thin noodles, they can break if handled roughly. Also, if a mass of precipitated DNA is pulled out of its surrounding liquid, it will clump together, much like cooked noodles will clump together when they are pulled out of their liquid.

Cheek Cell DNA Extraction: Laboratory Instructions

Capture Your Genetic Essence in a Bottle

Teacher's (Common) Station

Water bath at 50°C

Ice-cold bottle of 91% isopropanol or 95% ethanol on ice

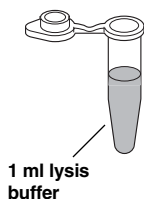
Students' Workstation (4 students per station)	Number required
Clear micro test tubes, each containing 1 ml lysis buffer	4
Blue micro test tube labeled "prot"	1
Pink micro test tube labeled "salt"	1
Clear, capless screw cap tubes	4
Assorted colored screw caps	4
Cytology brushes	8
5 ml round-bottom test tubes	4
Parafilm (small pre-cut pieces)	4
Disposable plastic transfer pipets	4
Foam micro test tube holder	1
Permanent marker	1
Disposable paper cup or beaker for waste collection	1

Procedure for DNA Extraction and Precipitation

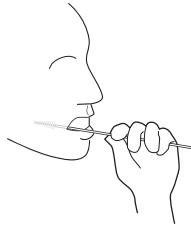
Steps 1 and 2: Collecting and Breaking Open Cells

To get as many cheek cells as possible, you will use two brushes to collect the cells from your mouth. You will combine the cells you get from both brushes into one tube of detergent solution. Ample cell collection is critical for success. For best results, make sure you spend the recommended amount of time collecting and carefully transferring the cells.

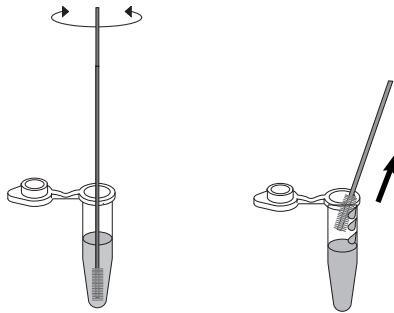
1. Obtain a clear micro test tube for yourself containing 1 ml of **lysis buffer**, and label it with your initials using a permanent marker.



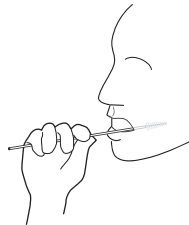
2. Take the first brush and roll the bristles firmly along the inside of your right cheek and in the space between your cheek and gum for 1 minute. **For best results, make sure you spend the recommended amount of time collecting the cells.** Brush firmly, but don't hurt yourself.



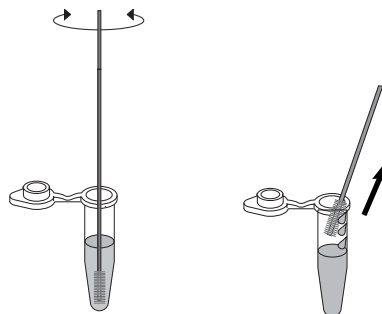
3. Place the brush with the cells into the tube containing lysis buffer. Swirl the brush around to release the cells from the brush into the buffer. Scrape the brush bristles across the top of the tube to transfer as much of the cells and liquid into the micro test tube as possible before disposing of your brush in the waste container.



4. Take a second, clean brush and collect cells from your left cheek, in between your cheek and gum, along the roof of your mouth, and under your tongue; again, try to collect as much cell material as possible.



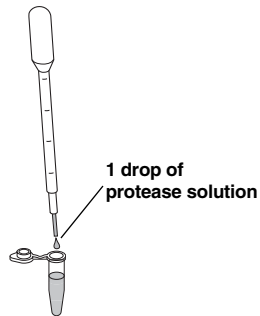
5. Place the brush with collected cells in the same tube as before, swirling the brush to release the cells. Scrape the brush bristles across the top of the tube to transfer as much of the cells and liquid into the micro test tube as possible before disposing of your brush in the waste container.



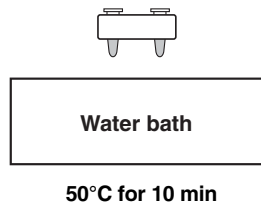
6. Cap the tube and gently invert it 5 times to mix.

Step 3: Removing proteins

1. Obtain the tube labeled “**prot**” and add 1 drop of protease solution to the microtube containing your cell extract. Cap the cell extract tube and gently invert it 5 times to mix.

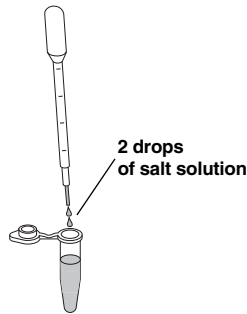


2. Place your cell extract tube in the foam micro test holder at your workstation and put the samples in a 50°C water bath (at the common workstation) for 10 minutes to allow the protease to work .

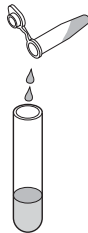


Steps 4 and 5: Making the DNA visible

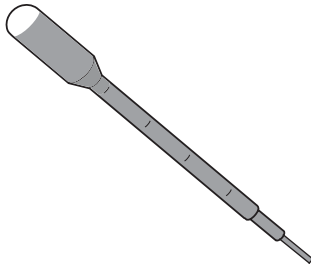
1. Remove your micro test tube from the water bath and add 2 drops of “**salt**” solution. Cap the tube and gently invert it 5 times to mix.



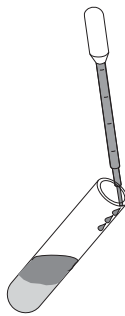
2. Label a 5 ml round-bottom test tube with your initials and transfer your cell extract into it.



- (You may need to do this step at the common workstation. Consult your teacher for specific instructions.) Fill a transfer pipet with cold alcohol.



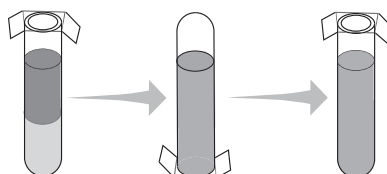
- Tilt the round-bottom tube at a 45° angle and slowly add the alcohol, carefully letting it flow gently down the inside of the tube. You should be able to see two layers (upper and lower) forming. As you add the alcohol, pay close attention to the place where the alcohol and cell extract layers meet. Write down your observations.



- Place your 5 ml tube upright either on the foam micro test tube holder or a test tube rack and **leave it undisturbed** at room temperature for 5 minutes.



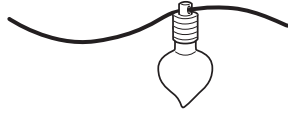
- After 5 minutes, look again at the contents of your tube, especially in the area where the alcohol and cell extract layers meet. Do you see anything? Write down your observations. Compare your sample with those of your classmates.
- Place a piece of Parafilm over the top of the tube, put your thumb over it, and mix by slowly inverting the tube 5 times. Look for any stringy, white or clear material. **This is your DNA!**



Student Manual: Basic Instruction

8. If you are going to make a DNA necklace, your teacher will provide you with a glass vial. With a plastic transfer pipet, carefully transfer the precipitated DNA along with approximately 750 μl to 1 ml of the alcohol solution into the vial. Then your teacher will help you seal the vial so you can complete the necklace.

If you are not going to make a DNA necklace, you can transfer and save your DNA in a screwcap tube. With a transfer pipet, gently withdraw your precipitated DNA along with about 500 μl of alcohol solution and transfer it into the screwcap tube. Tighten the cap and amaze your friends and family with your own DNA!



or



Student Manual: Advanced Instruction

Cheek Cell DNA Extraction Capture Your Genetic Essence in a Bottle

Contents

- Lesson 1** Introduction and background material
- Lesson 2** Cheek cell isolation, DNA extraction, and precipitation
- Lesson 3** DNA necklace preparation (optional)

Student Manual: Advanced Instruction

Cheek Cell DNA Extraction Capture Your Genetic Essence In a Bottle

Introduction

Deoxyribonucleic acid (DNA) is a molecule present in all living things, including bacteria, plants, and animals, and in almost all cell types. DNA is the carrier of genetic information and is responsible for determining a person's hair, skin, and eye color, facial features, complexion, height, blood type, and just about everything else that makes an individual unique. It also carries information required for cells to perform all of the functions that are common to all members of a species, or to all living things, and thus it is sometimes referred to as a biological "blueprint". Your personal blueprint is a combination of half of your mother's DNA (from her egg) and half of your father's DNA (from his sperm) during conception. All of your cells contain this complete set of instructions.

All DNA looks the same when it is extracted from cells, but it is exciting to look at your own DNA, knowing that this is really what makes you unique and alive. In this laboratory activity, you will extract your own DNA — a substance that holds your very own "blueprint" — from your cheek cells. You will use a quick and easy procedure that scientists routinely use to extract DNA from different organisms.

Every day scientists are making new discoveries as they study the information encoded in our DNA. Understanding DNA holds the possibility of curing diseases, the hope for millions who suffer from various genetic disorders and syndromes, making better products from biological sources, and even perhaps the key to longer life. We are beginning to understand who we are and why by studying our genetic material.

DNA Structure

At the molecular level, DNA looks like a twisted ladder or a spiral staircase. Two long molecules are aligned with each other, and the rungs are formed from pairs of chemical units called **bases**. This structure is referred to as a **double helix** because of the spiral, or helical form made by two strands. The bases function like letters in a code, so they are known as **A**, **G**, **T**, and **C** (abbreviations for their full names, adenine, guanine, thymine, and cytosine, respectively). Each base is connected to a sugar and a phosphate group, and the sugar and phosphate groups form the "backbones" of the ladder-like structure. (A **nucleotide** is one unit consisting of a base, sugar, and phosphate.) Scientists have found that **A** always pairs with **T**, and **G** always pairs with **C** in double-stranded DNA.



Fig. 3. A schematic representation of DNA (deoxyribonucleic acid). DNA is a long chainlike molecule that stores genetic information.

The 4 chemical letters of DNA are organized to make messages that can be understood by cells, called **genes**. These genes contain the information to make **proteins**, which are the basis for almost all of your body's structures and functions. Each of your cells contains several billion letters of DNA "text".

A DNA sequence is the particular arrangement or order of the bases along the DNA molecule. Human DNA sequences are 99.9% identical among each other. It is the <0.1% sequence variation that makes each of us unique. In other words, what makes you different from your classmate is an occasional difference in the sequence of bases in your genes.

The Genome, Chromosomes, Genes, DNA, RNA, and Proteins...What Is the Connection?

DNA is found within the nucleus of every cell in the human body, with the exception of mature red blood cells. The DNA is organized into structures called **chromosomes**, in which the long thin strands of DNA are tightly coiled around proteins. Every time a cell divides — for growth, repair, or reproduction — the chromosomes replicate in a highly organized process called mitosis. The 46 human chromosomes found in human cells are analogous to 46 volumes of an encyclopedia, which collectively contain all the information in your **genome**.

A **gene** is a section of DNA that contains the information to make a protein; it is like a written recipe that specifies the composition and order of assembly of a protein molecule. The human genome contains approximately 40,000 genes. The genome is analogous to a (gigantic) collection of cookbooks (remember, there are 46 "volumes" in the entire collection); not all of the recipes in a cookbook are prepared at once to make one meal, nor are all of the genes within the genome used in every cell. This selective gene expression according to cell type generates the characteristics of different cell types within your body. Basically, all of your cells contain the same books (chromosomes), but different cells read different recipes (genes) from the books.

Although genes specify the proteins that are made by cells, DNA is not the direct template for protein synthesis. The templates for protein synthesis are RNA (ribonucleic acid) molecules called messenger RNA (mRNA). Each mRNA molecule is simply a copy of the DNA sequence from one gene. mRNAs are the intermediates that carry the information from the DNA within the nucleus to the **ribosomes**, or protein manufacturers, within the cytoplasm. The ribosomes decode the genetic information and link together the appropriate amino acids to make the **protein** that is encoded by the gene. All the proteins made within a cell function to give the cell its traits.

Focus questions:

1. Imagine you are trying to explain the difference between chromosomes, genes, and DNA to your younger brother or sister who is two years younger than you. Write down your explanation in simple words that they could understand.

2. Does a liver cell contain the same chromosomes as a cheek cell?

3. If you wanted to isolate a copy of the gene that codes for a protein found in the stomach, could that gene be located in cheek cells? Explain your reasoning.

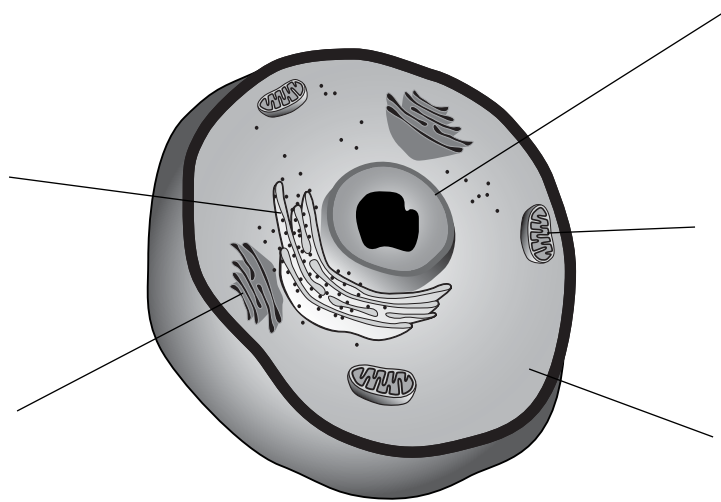
How can DNA be isolated from cells?

Step 1. Collecting cells

The first step in DNA isolation is the collection of cells. The lining of the mouth is a good source of cells. These cells divide very often and are continually being sloughed off, making them an accessible source of cells. Simply scraping the inside of your mouth gently and thoroughly with a brush allows you to collect a quantity of cells from which you can isolate your own DNA.

Focus questions:

Below is a schematic image of a cheek cell.



4. Label the cellular compartments, including the cell membrane, cytoplasm, and nucleus.
5. In which cellular compartment do you expect to find your genomic DNA?
6. Why is an intermediate like mRNA needed to copy the information from the genomic DNA so it can be translated into proteins?
7. What do you think will be the first step in isolating DNA from your cells?

Step 2. Lysing the cells and dissolving the phospholipid bilayer membranes

If you guessed that the first step of DNA extraction is to break open the cells, you are right! Detergent dissolves oil-based molecules, and the cell and nuclear membranes are mainly oil-based (you may have already heard of cell membranes being composed of “phospholipid bilayers”). After scraping cells from your cheeks, you will put the cells into a solution that contains detergent.

Focus questions:

8. Once the membranes have been dissolved, the DNA is released into the solution, but so are many other types of cellular molecules. List some types of molecules besides DNA that you would expect to find in a cell.
9. What method or agent do you think might be used to break down these unwanted molecules?

Step 3. Using protease to break down cellular proteins

As you may have already guessed, the most prevalent class of molecules that would interfere with the precipitation of pure DNA is protein. We can easily get rid of protein without damaging the DNA by using a specific enzyme that digests proteins, called a protease. Protease breaks the peptide bonds between the amino acids of proteins. By destroying all the proteins you will also eliminate DNases, enzymes that digest DNA (because enzymes are proteins).

Focus questions:

10. What proteins might be associated with DNA in the cell?
11. The protease used in this procedure functions best at 50°C. Would you expect this enzyme to be isolated from *E. coli* bacteria? Explain your answer. **Hint:** Where does *E. coli* live?

12. Meat tenderizer is often used to tenderize tough pieces of meat, like steak. Knowing that steak is made of protein-rich muscle tissue from cows, can you think of an explanation for how meat tenderizer works?

Step 4. Making DNA insoluble

You will add a salt solution to your sample, which will cause the DNA to become less soluble in the cell extract. DNA has a negative electrical charge due to the phosphate groups on the DNA backbone. When the salt is added, the positively charged sodium ions of the salt are attracted to the negative charges of the DNA, neutralizing the electrical charge of the DNA. This allows the DNA molecules to come together instead of repelling each other.

Step 5. Precipitating the DNA with cold alcohol

To separate the DNA from the other molecules in the cell extract, you will add cold alcohol to your sample. Upon the addition of cold alcohol, the DNA will precipitate because it is less soluble in alcohol than in water. The colder the ethanol is, the less soluble the DNA will be in it. This is similar to the solubility of sugar in tea (or any drink); sugar dissolves more readily in hot tea than in iced tea.

In the presence of high salt and cold alcohol, the DNA that had been released from your cells precipitates and aggregates until it can be seen with the naked eye! The other molecules in the cell extract, such as the amino acids and carbohydrates, remain dissolved in the alcohol and water and will not be visible. It takes many thousands of strands of DNA to form a fiber large enough to be visible. Each strand will have thousands of genes on it, so you will be looking at material that contains millions of genes at once. Remember, though, that you are seeing the DNA from many thousands of cells all together.

Focus questions:

13. Match the outcomes on the left with the laboratory steps on the right.

- | | |
|------------------------------------|--|
| ___ Harvest the cells | A. Scrape a brush against the inside of your cheek |
| ___ Dissolve cell membranes | B. Add protease, incubate at 50°C |
| ___ Precipitate the DNA | C. Mix in a detergent solution |
| ___ Break down proteins | D. Layer cold alcohol over cell extract |
| ___ Make DNA less soluble in water | E. Add salt |

Cheek Cell DNA Extraction: Laboratory Instructions

Capture Your Genetic Essence In a Bottle

Teacher's (Common) Station

Water bath at 50°C

Ice-cold bottle of 91% isopropanol or 95% ethanol on ice

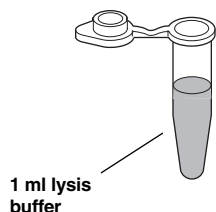
Students' Workstation (4 students per station)	Number required
Clear micro test tubes, each containing 1 ml lysis buffer	4
Blue micro test tube labeled "prot"	1
Pink micro test tube labeled "salt"	1
Clear, capless screw cap tubes	4
Assorted colored screw caps	4
Cytology brushes	8
5 ml round-bottom test tubes	4
Parafilm (small pre-cut pieces)	4
Disposable plastic transfer pipets	4
Foam micro test tube holder	1
Permanent marker	1
Disposable paper cup or beaker for waste collection	

Procedure for DNA Extraction and Precipitation

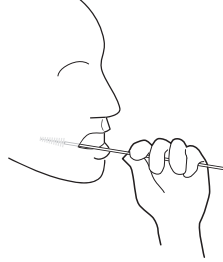
Steps 1 and 2: Collecting and breaking open cells

To get as many cheek cells as possible, you will use two brushes to collect the cells from your mouth. You will combine the cells you get from both brushes into one tube of detergent solution. Ample cell collection is critical for success. For best results, make sure you spend the recommended amount of time collecting and carefully transferring the cells.

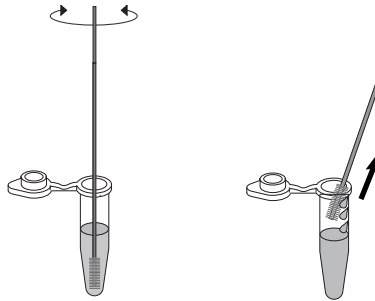
1. Obtain a clear micro test tube for yourself containing 1 ml of **lysis buffer**, and label it with your initials using a permanent marker.



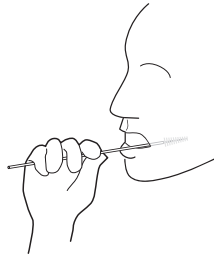
2. Take the first brush and roll the bristles firmly along the inside of your right cheek and in the space between your cheek and gum for 1 minute. **For best results, make sure you spend the recommended amount of time collecting the cells.** Brush firmly, but don't hurt yourself.



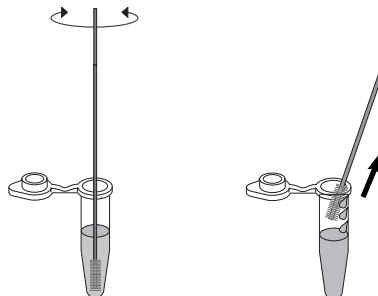
3. Place the brush with the cells into the tube containing lysis buffer. Swirl the brush around to release the cells from the brush into the buffer. Scrape the brush bristles across the top of the tube to transfer as much of the cells and liquid into the micro test tube as possible before disposing of your brush in the waste container.



4. Take a second, clean brush and collect cells from your left cheek, in between your cheek and gum, along the roof of your mouth, and under your tongue; again, try to collect as much cell material as possible.



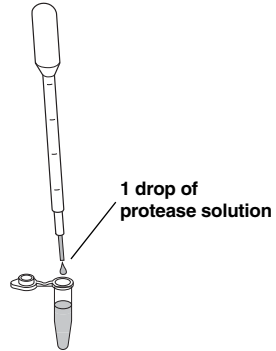
5. Place the brush with collected cells in the same tube as before, swirling the brush to release the cells and removing as much liquid as possible before disposing of the brush.



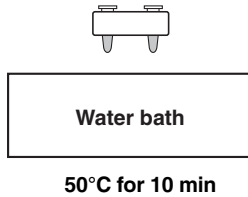
6. Cap the tube and gently invert it 5 times to mix.

Step 3: Removing proteins

1. Obtain the tube labeled “**prot**” and add 1 drop of protease solution (35 μ l if you are using an adjustable micropipetor) to the micro tube containing your cell extract. Cap the cell extract tube and gently invert it 5 times to mix.

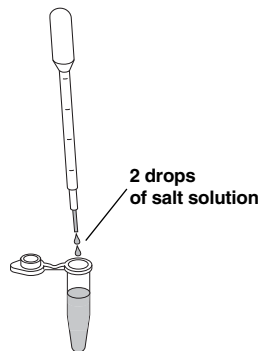


2. Place your cell extract tube in the foam micro test tube holder at your workstation and put the samples in a 50°C water bath (at the common workstation) for 10 minutes to allow the protease to work.

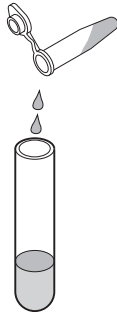


Steps 4 and 5: Making the DNA visible

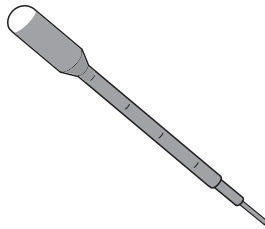
1. Remove your micro test tube from the water bath and add 2 drops (70 μ l if you are using an adjustable micropipetor) of “**salt**” solution. Cap the tube and gently invert it 5 times to mix.



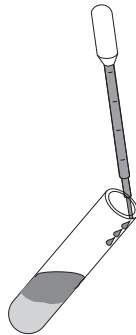
- Label a 5 ml round-bottom test tube with your initials and transfer your cell extract into it.



- (You may need to do this step at the common workstation. Consult your teacher for specific instructions.) Fill a transfer pipet with cold alcohol.



- Tilt the round-bottom tube at a 45° angle and slowly add the alcohol, carefully letting it flow gently down the inside of the tube. You should be able to see two layers (upper and lower) forming. As you add the alcohol, pay close attention to the place where the alcohol and cell extract layers meet. Write down your observations.

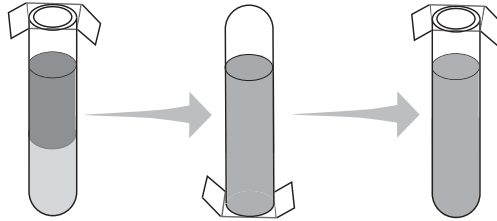


- Place your 5 ml tube upright either on the foam micro test tube holder or a test tube rack and **leave it undisturbed** at room temperature for 5 minutes.



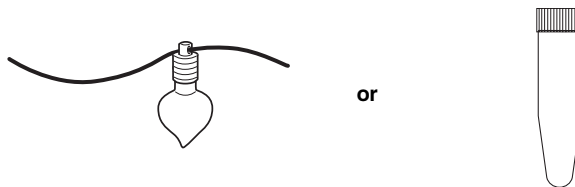
- After 5 minutes look again at the contents of your tube, especially in the area where the alcohol and cell extract layers meet. Do you see anything? Write down your observations. Compare your sample with those of your classmates.

7. Place a piece of Parafilm over the top of the tube, put your thumb over it, and mix by inverting the tube 5 times. Look for any stringy, white or clear material. **This is your DNA!**



8. If you are going to make a DNA necklace, your teacher will provide you with a glass vial. With a plastic transfer pipet, carefully transfer the precipitated DNA along with approximately 750 μ l to 1 ml of the alcohol solution into the vial. Then your teacher will show you how to seal the vial so you can complete the necklace.

If you are not going to make a DNA necklace, you can transfer and save your DNA in a screwcap tube. With a transfer pipet, gently withdraw your precipitated DNA along with about 500 μ l of alcohol solution and transfer it into the screwcap tube. Tighten the cap and amaze your friends and family with your own DNA!



Extension Activities

Dry laboratory demonstration of DNA extraction

For students in 5th through 8th grades, we recommend a dry laboratory demonstration of the DNA extraction procedure to help students visualize what is happening on the molecular level during each step. This is a fun and visual exercise that will allow teachers to present the concepts needed to make this laboratory more meaningful. To demonstrate the process of DNA isolation from cheek cells, you can create a model of a cell using a clear latex balloon filled with various small items and string to represent membranes, organelles, protein, and DNA. Emphasize that detergent dissolves membranes (breaking open the balloon), protease digests proteins (crushing small items), and salt and alcohol cause the DNA to precipitate and aggregate (gathering of string).

Microscopic observation and nuclear staining of cheek cells

Before transferring their cheek cells to the micro test tubes containing lysis buffer, have your students gently touch the brush to a drop of water on a microscope slide to smear some cells onto it. During the 10-minute incubation with the protease, add one drop of nuclear stain, such as Bio-Rad's Bio-Safe DNA stain (166-0400EDU), to the slide and observe the cells under low and medium magnifications. (Students should have had prior instruction in proper use of a microscope.) Have students make observations and draw sketches, labeling visible cell structures. Students could also view a drop of their cell suspension after the incubation, noting any differences in the samples before and after the lysis and digestion steps.

Answers to Focus Questions (Basic Instruction)

- 1. How could you test whether you were actually collecting cells from your cheeks? What piece of laboratory equipment might you use?**

You could touch your brush to a glass microscope slide after collecting your cheek cells and look at them under a microscope.

- 2. When washing dishes, what works better, warm or cold water? Which do you think will help the detergent break open the cell, warm or cold temperature?**

Warm water works better when washing dishes because it helps make the fats and proteins dissolve better in dish detergent. Warm temperature will help the detergent in the lysis buffer break open the cells.

- 3. Do you think your DNA will be visible after you have broken open your cells? Why or why not?**

Your DNA will not be visible after you have broken open your cells. It will be dissolved in the lysis buffer.

- 4. Where do you think you would find proteases in your body? Hint: Where do the proteins that you eat get broken down?**

Proteases are found in your stomach, where the proteins that you eat get digested.

- 5. Have you ever tried to add sugar to iced tea? Does the sugar dissolve easily? How does this compare to dissolving the same amount of sugar in the same amount of hot tea?**

Sugar dissolves much less easily in iced tea than in hot tea. The cold temperature of the iced tea reduces the sugar's solubility, or ability to dissolve. In general, heat increases the solubility of substances dissolved in liquid.

Answers to Focus Questions (Advanced Instruction)

1. **Imagine you are trying to explain the difference between chromosomes, genes, and DNA to your younger brother or sister who is two years younger than you. Write down your explanation in simple words that they could understand.**

DNA is a chemical found in all living things and is passed from parents to children. It carries the information needed to make you who you are.

Chromosomes are long strands of coiled DNA. The DNA within your cells is organized into structures called chromosomes, which make it easy to store within the cell and to copy when cells divide.

Genes are sections of DNA that contain the information needed to make proteins, which perform critical jobs within living cells.

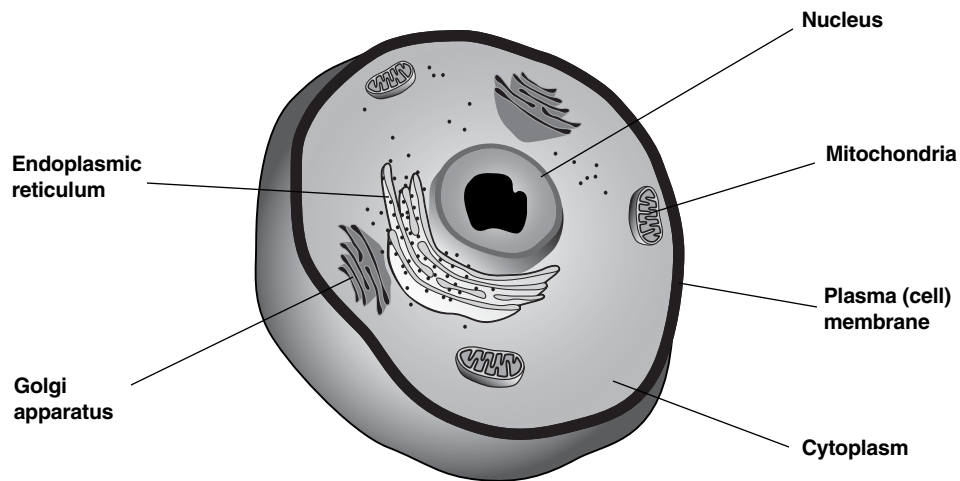
2. **Does a liver cell contain the same chromosomes as a cheek cell?**

Yes. The genomic DNA found in all nonreproductive cells is the same, no matter what tissue the cells come from.

3. **If you wanted to isolate a copy of the gene that codes for a protein found in the stomach, could that gene be located in cheek cells? Explain your reasoning.**

The gene that codes for a stomach protein would be found in the genomic DNA inside a cheek cell. However, the cheek cell would not make the messenger RNA, or copies, of the gene for the stomach protein. Stomach protein genes are expressed only in the stomach.

Below is a schematic image of a cheek cell.



4. **Label the cellular compartments, including the cell membrane, cytoplasm, and nucleus.**
5. **In which cellular compartment do you expect to find your genomic DNA?**

Genomic DNA is located in the nucleus.

6. Why is an intermediate like mRNA needed to copy the information from the genomic DNA so it can be translated into proteins?

Genomic DNA is in the nucleus and always remains there (like an archived book that can never leave a library), but the protein-making ribosomes are in the cytoplasm. A mobile intermediate is needed to bring the genetic information from the nucleus to the cytoplasm.

7. What do you think will be the first step in isolating DNA from your cells?

The cell and nuclear membranes must be disrupted to release the DNA.

8. Once the membranes have dissolved, the DNA is released into the solution, but so are many other types of cellular molecules. List some types of molecules besides DNA that you would expect to find in a cell.

Proteins, lipids, sugars, and minerals (salts) are common cell components.

9. What method or agent do you think might be used to break down these unwanted molecules?

There are enzymes that specifically digest all kinds of biological molecules. Proteases break down proteins, detergents dissolve lipids, and enzymes like beta-galactosidase break down sugars. Heat and agitation can speed up these digestion processes.

10. What proteins might be associated with DNA in the cell?

Chromosomal DNA is bound by histones. Other associated nuclear proteins may include DNA polymerase or transcription factors.

11. The protease used in this procedure functions best at 50°C. Would you expect this enzyme to be isolated from *E. coli* bacteria? Explain your answer. Hint: Where does *E. coli* live?

No. *E. coli*, which lives in our gut, thrives around our body temperature, 37°C. An enzyme whose optimal temperature is 50°C was probably isolated from an organism that lives at or near that temperature.

12. Meat tenderizer is often used to tenderize tough pieces of meat, like steak. Knowing that steak is made of protein-rich muscle tissue from cows, can you think of an explanation for how meat tenderizer works?

Many meat tenderizers contain papain, which is a protease. The protease breaks down the protein molecules. By partially degrading some of the proteins, the tough muscle/meat is made softer and more tender.

13. Match the outcomes on the left with the laboratory steps on the right.

- | | |
|--|--|
| <u> </u> A Harvest the cells | A. Scrape a brush against the inside of your cheek |
| <u> </u> C Dissolve cell membranes | B. Add protease, incubate at 50°C |
| <u> </u> E Precipitate the DNA | C. Mix in a detergent solution |
| <u> </u> B Break down proteins | D. Layer cold alcohol over cell extract |
| <u> </u> D Make DNA less soluble in water | E. Add salt |

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BIO-RAD**Bio-Rad
Laboratories, Inc.****Life Science
Group**

Web site www.bio-rad.com **Bio-Rad Laboratories Main Office** 2000 Alfred Nobel Drive, Hercules, CA 94547, Ph. (510) 741-1000, Fx. (510) 741-5800
Also in: **Australia** Ph. 02 9914 2800, Fx. 02 9914 2889 **Austria** Ph. (01) 877 89 01, Fx. (01) 876 56 29 **Belgium** Ph. 09-385 55 11, Fx. 09-385 65 54
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