



# **Biotechnology Explorer™**

## **Genes in a Bottle™ Kit DNA Extraction Module**

**Catalog #166-2000EDU**

**DNA Helix Necklace Module  
(catalog #166-2250EDU)  
must be purchased separately.**

**[explorer.bio-rad.com](http://explorer.bio-rad.com)**

**See individual components for storage temperature.**

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Biotechnology Explorer kit curriculum.

**BIO-RAD**

## Capture Your Essence!

**Bottle your DNA!** 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 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 retaining intact DNA. The DNA is finally collected by precipitation in a form that can be manipulated as desired.

With this simple lab activity, students gain practical knowledge by conducting a real-world 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. The DNA strands are then easily collected and transferred to a helix vial, and the vial is fashioned into a necklace!

**Seeing is believing.** 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 and helps students comprehend this previously invisible substance of life.

**Learning opportunities for all levels of instruction.** This activity is designed for any classroom environment and requires no specialized equipment or stains. For middle school students, it's a perfect introduction to the exciting world of DNA science. 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. We welcome your comments and suggestions. Have fun!

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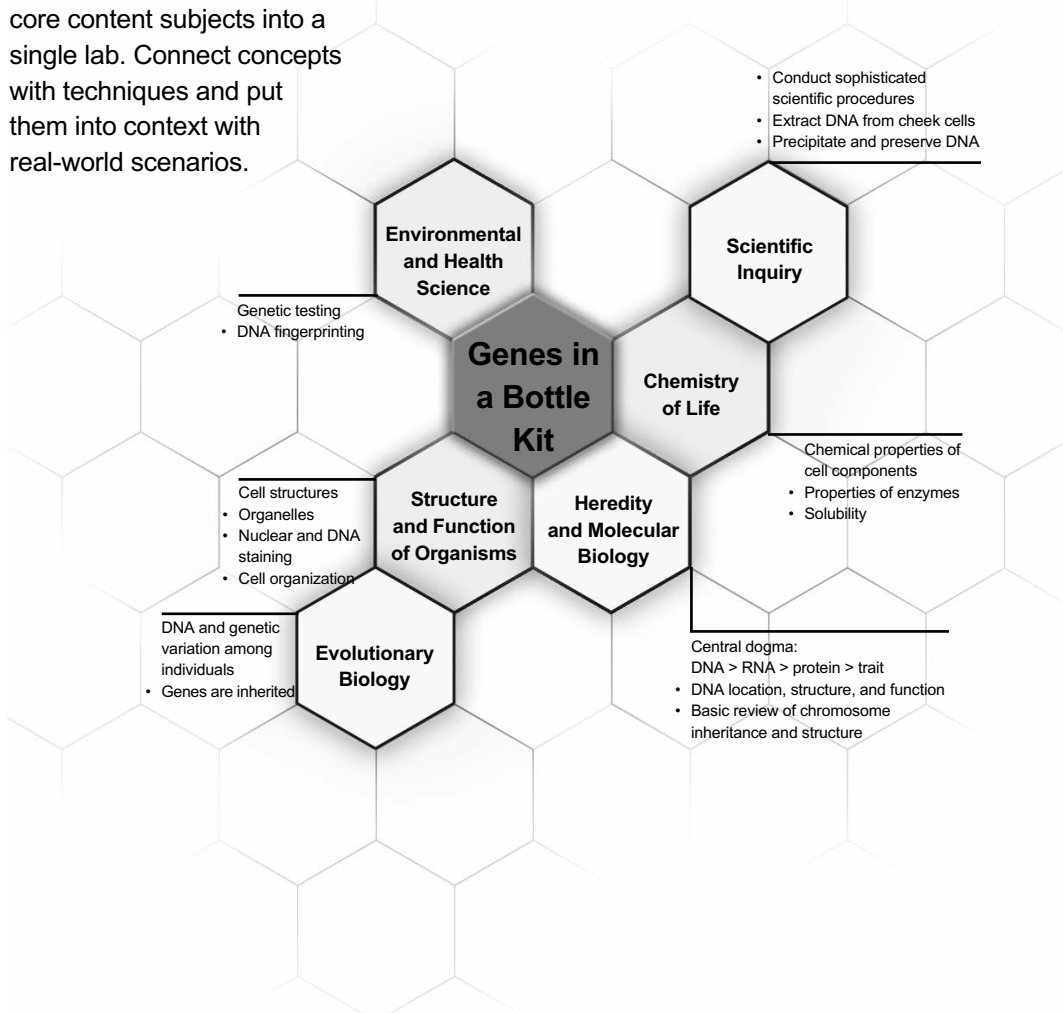
**Create context. Reinforce learning. Stay current.**

New scientific discoveries and technologies create more content for you to teach, but not more time. Biotechnology

Explorer kits help you teach more effectively by integrating multiple

core content subjects into a single lab. Connect concepts with techniques and put

them into context with real-world scenarios.



# Table of Contents

## Teacher's Guide

<b>Kit Inventory Checklist</b> .....	1
<b>Overview for the Teacher</b> .....	2
Why Should You Teach DNA Extraction? .....	2
Intended Audience .....	2
Curriculum Fit .....	3
Recommended Student Background .....	3
Activity Timeline .....	3
Safety Issues .....	3
Keys to Success .....	3
Volume Measurements .....	3
Background and Fundamentals for Basic Level Instruction .....	4
Background and Fundamentals for Advanced Level Instruction .....	6

## Teacher's Laboratory Guide

Implementation Timeline .....	8
Teacher's Advance (Pre-Laboratory) Preparation .....	8
Workstation Checklist .....	10
Quick Guide for DNA Extraction and Precipitation .....	11

## Student Manuals

<b>Basic Level Student Manual</b> .....	13
Introduction .....	14
Workstation Checklist .....	17
Procedure for DNA Extraction and Precipitation .....	17
<b>Advanced Level Student Manual</b> .....	21
Introduction and Focus Questions .....	22
Workstation Checklist .....	27
Procedure for DNA Extraction and Precipitation .....	27

## Extension Activities

Dry Laboratory Demonstration of DNA Extraction .....	30
Microscopic Observation and Nuclear Staining of Cheek Cells .....	30
Staining precipitated DNA .....	31
<b>Answers to Focus Questions</b> (Basic Instruction) .....	32
<b>Answers to Focus Questions</b> (Advanced Instruction) .....	33

## Teacher's Guide

### Kit Inventory Checklist

This section lists the components provided in this Genes in a Bottle Kit. It also lists required and optional accessories. Each kit contains sufficient materials to outfit 9 student workstations of up to four students per workstation. Use this checklist to inventory your supplies before beginning advanced preparation.

<b>Kit Components</b>	<b>Quantity</b>	<input checked="" type="checkbox"/>
Lysis buffer	110 ml	<input type="checkbox"/>
Powdered protease and salt	1.5 g	<input type="checkbox"/>
15 ml conical tubes	50	<input type="checkbox"/>
Colored 1.5 ml microcentrifuge tubes	60	<input type="checkbox"/>
Disposable plastic transfer pipets	60	<input type="checkbox"/>
<b>Required Accessories (not included in this kit)</b>	<b>Quantity</b>	<input checked="" type="checkbox"/>
91% isopropanol (available at drug stores) or 95% ethanol	approx. 360 ml	<input type="checkbox"/>
Water bath with thermometer, set at 50°C*	1	<input type="checkbox"/>
Permanent markers	1–9	<input type="checkbox"/>
Container of ice	1	<input type="checkbox"/>
Disposable paper cups or beakers for waste disposal	9	<input type="checkbox"/>
Beaker or rack to hold 15 ml tubes in water bath (need space for 36 tubes maximum)	1	<input type="checkbox"/>
Microcentrifuge tube racks (catalog #166-0481EDU)	1–9	<input type="checkbox"/>

\* If a temperature-controlled water bath is not available, use one or more insulated containers (Styrofoam is best) large enough to hold a beaker or rack containing up to 36 15 ml tubes, and fill with water heated to 50°C.

### **Optional DNA Helix Necklace Module\*\* (catalog # 166-2250EDU not included in this kit)**

\*\*Each DNA helix necklace module contains enough material to prepare 36 necklaces,  
**Catalog #166-2250EDU contains:**

Helix vials	36
Screw caps	36
Cords	36

### Refills Available Separately

<b>Catalog #</b>	<b>Description</b>
166-2300EDU	<b>Genes in a Bottle Kit</b> , contains (1) DNA Extraction Module and (1) DNA Helix Necklace Module. Serves up to 36 students
166-2000EDU	<b>Genes in Bottle DNA Extraction Module</b> (serves 36 students)
166-2250EDU	<b>Genes in a Bottle DNA Helix Necklace Module</b> (serves 36 students)
166-2001EDU	<b>Genes in a Bottle DNA Extraction Refill Package</b> , includes lysis buffer and powdered protease + salt
166-2002EDU	<b>Genes in a Bottle Lysis Buffer</b> , 110 ml
166-0480EDU	<b>Disposable plastic transfer pipets</b> , nonsterile, 500
166-0473EDU	<b>Colored 1.5 ml microcentrifuge tubes</b> , 6 colors, 600
166-0475EDU	<b>Conical centrifuge tubes</b> , 15 ml, 50

# 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 helix 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 45-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

In this experiment, no special biosafety handling is required. There is no greater risk of exposure to infectious agents in this activity than in normal student interactions (sharing a beverage, sneezing). Students will handle their own biological samples. Lysis buffer is added to break open the cells, rendering them inviable.

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.

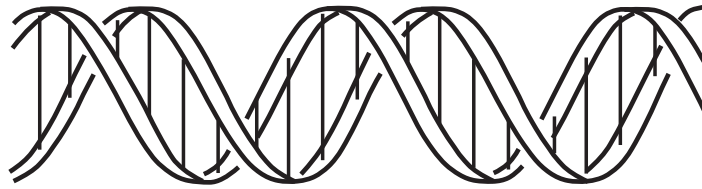
### Where is DNA found?

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. 1. 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 gently chewing the insides of their cheeks to loosen cells from the inside of their mouth then rinsing their mouths with water to collect the cells. Lysis buffer is then added to the solution of cells. 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.

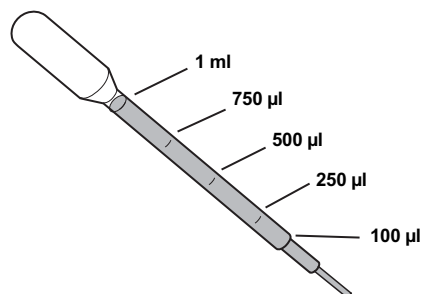
### Implementation Timeline

1–2 days	Lesson 1	Introduction and background material <b>Optional</b> dry laboratory demonstration of DNA extraction — recommended for students in grades 5–8. See extension activities at the end of the manual.
45 minutes	Lesson 2	Cheek cell isolation, DNA extraction, and precipitation
30–45 minutes	Lesson 3	<b>Optional</b> DNA necklace preparation

### Teacher's Advance (Prelaboratory) Preparation

#### 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.
- Take the pouch containing the powdered protease + salt ('**prot**') and cut open one corner. Pour the powder into one of the 15 ml tubes and label this ('**prot**'). Add 15 ml of water to the **prot**. Drinking water works well; distilled water, as used in laboratories, may be acceptable.

Once the **prot** is rehydrated, it is good for up to a week if stored in a refrigerator, at 4°C. If you plan to use the kit for several groups of students over a few weeks, it is recommended that you measure out some of the protease for use now, and rehydrate the remaining protease for use later. The protease should be rehydrated at a concentration of 100 mg/ml.

Aliquot 1.25 ml of the rehydrated **prot** into 8 pink micro test tubes as described below.

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

1. For each student, dispense 3 ml of water into a 15 ml tube (up to 4 tubes per station). Any type of drinking water is acceptable.
2. Dispense 1.25 ml of the rehydrated protease + salt (see p. 8 for dilution instructions) into 9 pink microcentrifuge tubes and label the tubes '**prot**'.
3. Dispense 10 ml of lysis buffer into 9 x 15 ml tubes. Label each tube '**lysis**'.
4. Place 4 x 15 ml tubes of water and one tube of lysis buffer in a cup or test tube holder, and 1 pink microcentrifuge tube labeled '**prot**' on the lab bench or in a microcentrifuge tube rack at each student workstation.

**Note:** Some users may find collecting mouthwash in 15 ml tubes difficult. As an alternative, instructors may elect to use a small drinking cup to dispense water and collect mouthwash.

# 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 with a beaker or rack that can hold up to 36 x 15 ml tubes

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

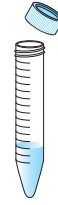
<b>Students' Workstation (4 students per station)</b>	<b>Number Required</b>
15 ml tubes, each containing 3 ml water	4
Pink microcentrifuge tube labeled ' <b>prot</b> ', containing 1.25 ml of protease + salt	1
15 ml tube labeled ' <b>lysis</b> ' containing 10 ml lysis buffer	1
Disposable plastic transfer pipets	6
Permanent marker	1
Disposable paper cup or beaker for holding 15 ml tubes and subsequent 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 mouth cells.

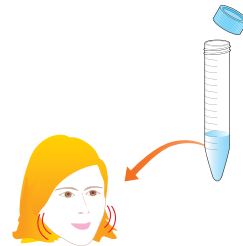
## Quick Guide for DNA Extraction and Precipitation

1. Obtain 15 ml tube containing 3 ml water from your instructor. Label the tube with your initials.



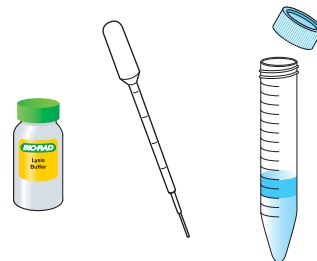
2. Gently chew the insides of your cheeks for 30 seconds. It is NOT helpful to draw blood!

3. Take the water from the 15 ml tube into your mouth, and swish the water around vigorously for 30 seconds.



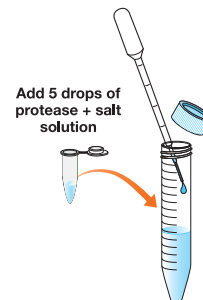
4. Carefully expel the liquid back into the 15 ml tube.

5. Obtain the tube of lysis buffer from your workstation, and add 2 ml of lysis buffer to your tube.

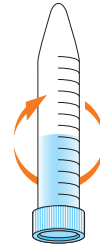


6. Place the cap on the tube, and gently invert the tube 5 times (don't shake your tube!). Observe your tube — do you notice any changes? If you do, write them down.

7. Obtain the tube of protease (**prot**) at your workstation. Add 5 drops of protease to your tube.



8. Place the cap on your tube, and gently invert it a few times.

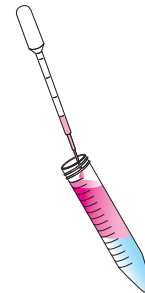


9. Place your tube in a test tube rack or beaker in the water bath and incubate at 50°C for 10 minutes. Remove your tubes from the water bath.



50°C for 10 min

10. Obtain the tube of cold alcohol from your instructor or at the common workstation. Holding your tube at a 45° angle, fill your tube with cold alcohol, by adding approximately 10 ml to your tube. It will take repeated additions to add 10 ml of the cold alcohol using the disposable plastic transfer pipet.

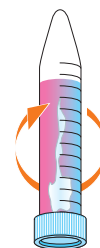


11. Place your cap on your tube, and let it sit undisturbed for 5 minutes. Write down anything you observe happening in the tube.



12. After 5 minutes, slowly invert the tube 5 times to help the DNA, which has begun to precipitate, to aggregate.

13. With a disposable plastic transfer pipet, carefully transfer the precipitated DNA along with approximately 500  $\mu$ l of the alcohol solution into a small glass vial provided in the DNA helix necklace module (cat #166-2250EDU). If you are not going to make a DNA necklace, save your DNA in a 1.5 ml microcentrifuge tube provided in this kit.





## **Student Manual: Basic Instruction**

### **Cheek Cell DNA Extraction Capture Your Genetic Essence in a Bottle**

#### **Contents**

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

# Student Manual: Basic Instruction

## Lesson 1

### 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 all the information needed to make a protein.

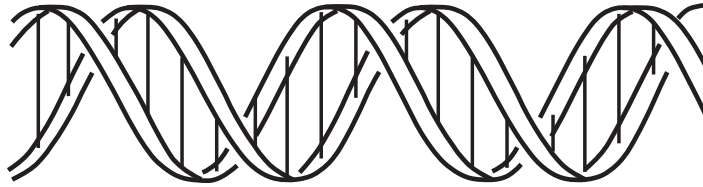
Your DNA sequence is the particular arrangement or order of the chemical letters within your complete DNA collection, or **genome**. Scientists have determined that human DNA sequences are 99.9% identical. It is the <0.1% sequence variation from person to person that makes each of us unique. In other 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 epithelial cheek 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 gently chewing your cheeks and rinsing your mouth with water. The cells that line your mouth divide once or twice a day. Old cells fall off your cheeks continuously as new cells replace them. In fact, your cheek 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

## Lesson 2

### Capture Your Genetic Essence in a Bottle

#### Workstation Checklist

##### Teacher's (Common) Station

Water bath at 50°C

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

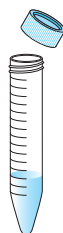
<b>Students' Workstation (4 students per station)</b>	<b>Number required</b>
15 ml tubes, each containing 3 ml water	4
Pink microcentrifuge tube labeled 'prot', containing 1.25 ml of protease + salt	1
15 ml tube labeled 'lysis' containing 10 ml lysis buffer	1
Disposable plastic transfer pipets	6
Permanent marker	1
Disposable paper cup or beaker for holding 15 ml tubes and subsequent waste collection	1
Microcentrifuge tube rack (optional for holding microcentrifuge tube with protease + salt solution)	1

#### Procedure for DNA Extraction and Precipitation

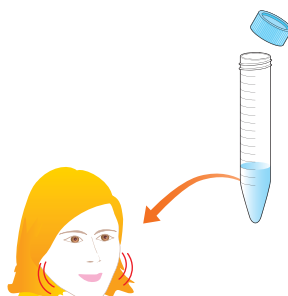
##### Steps 1 and 2: Collecting and Breaking Open Cells

To collect as many cheek cells as possible, you will gently chew the insides of your mouth for 30 seconds and then rinse your mouth with a small amount of water. Ample cell collection is critical for success. For best results, make sure you spend the recommended amount of time collecting the cells.

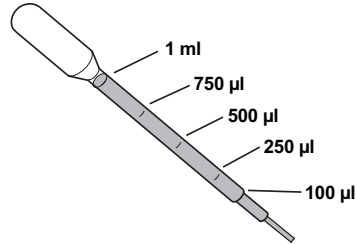
1. Obtain a 15 ml tube containing 3 ml of water, and label it with your initials.



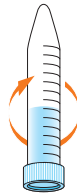
2. Gently chew the insides of your mouth for 30 seconds.
3. Take the 3 ml of water from your tube into your mouth and rinse vigorously for 30 seconds. Don't swallow the water!



- Carefully expel all your water mouthwash back into your 15 ml tube.
- Locate the 15 ml tube at your workstation labeled '**lysis**'. Using a fresh disposable plastic transfer pipet, add 2 ml of lysis buffer to your tube.

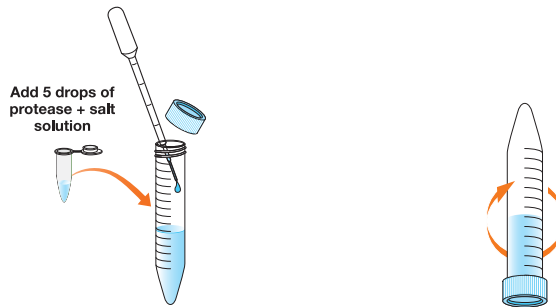


- Place the cap back on your tube. Gently invert your tube 5 times to lyse your cells. Don't shake the tube. If you observe any changes to your cells at this time, write them down.



### Step 3: Removing proteins

- Obtain the pink microcentrifuge tube labeled '**prot**' and add 5 drops of protease and salt solution to the 15 ml tube containing your cell extract. Cap the cell extract tube and gently invert it 5 times to mix.



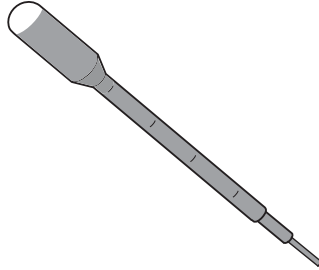
- Place your cell extract tube in the beaker or test tube holder in the 50°C water bath (at the common workstation) for 10 minutes to allow the protease to work.



50°C for 10 min

#### Steps 4 and 5: Making the DNA visible

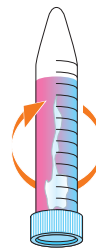
1. (You may need to do this step at the common workstation. Consult your teacher for specific instructions.) Fill a disposable transfer pipet with cold alcohol.



2. Obtain the tube of cold alcohol from your instructor or at the common workstation. Add 10 ml of the alcohol to your tube as follows. Hold your tube at a 45° angle and add the alcohol by slowly dispensing it down the inside wall of the tube. It will take repeated additions to add 10 mls. Screw the cap back onto your tube.

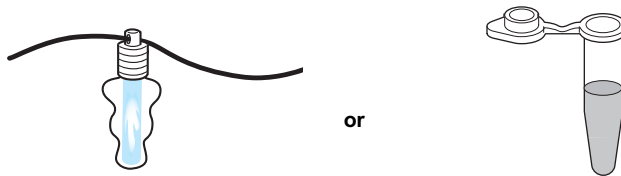


3. Place your 15 ml tube upright either in the cup or a test tube rack and **leave it undisturbed** at room temperature for 5 minutes.
4. After 5 minutes, look again at the contents of your tube, especially in the area where the alcohol and cell extract layers meet. Do you see anything? Write down your observations. Compare your sample with those of your classmates.
5. With the cap of your tube tightly sealed, mix the contents of your tube by slowly inverting the tube 5 times. Look for any stringy, white or clear material. **This is your DNA!**



6. If you are going to make a DNA necklace, your teacher will provide you with a helix vial, screw cap, and cord. With a disposable transfer pipet, carefully transfer the precipitated DNA along with approximately 500  $\mu$ l of the alcohol solution into the vial, screw on the cap tightly.

If you are not going to make a DNA necklace, you can transfer and save your DNA in a 1.5 ml microcentrifuge tube. With a disposable plastic transfer pipet, gently withdraw your precipitated DNA along with about 1 ml of alcohol solution and transfer it into the microcentrifuge tube. Tighten the cap and amaze your friends and family with your own DNA!





## **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

## Lesson 1

### 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.

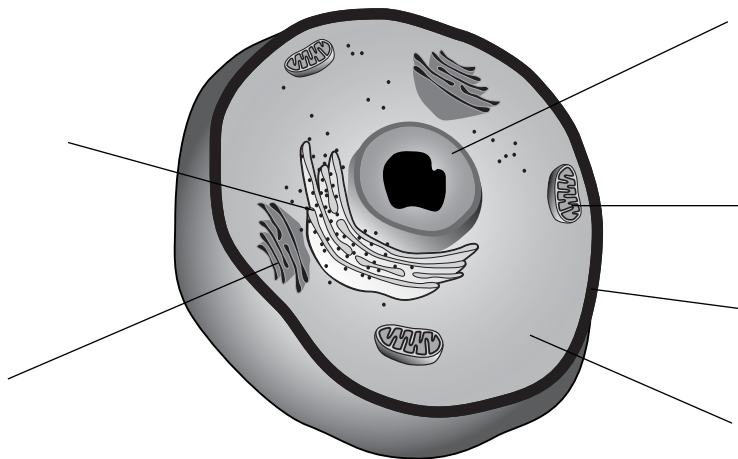
**How can DNA be isolated from cells?**

**Step 1. Collecting epithelial cheek 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 by gently chewing your cheeks your cheeks and rinsing the inside of your mouth thoroughly with water 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 hydrophobic (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 collecting cells from your cheeks, you will add 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

The protease solution that you added to your sample also contains salt. The salt will cause the DNA to become less soluble in solution. 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 or aggregate 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. Gently chew the insides of your mouth and then rinse vigorously with water

\_\_\_ 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

## Lesson 2

### Capture Your Genetic Essence In a Bottle

#### Workstation Checklist

##### 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
--	-----------------

15 ml tubes, each containing 3 ml water	4
---	---

Pink microcentrifuge tube labeled 'prot', containing 1.25 ml protease + salt	1
--	---

15 ml tube labeled 'lysis' containing 10 ml lysis buffer	1
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Disposable plastic transfer pipets	6
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Permanent marker	1
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Disposable paper cup or beaker for holding 15 ml tubes and subsequent waste collection	1
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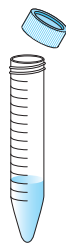
Microcentrifuge tube rack (optional for holding microcentrifuge tube with protease + salt solution)	1
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#### Procedure for DNA Extraction and Precipitation

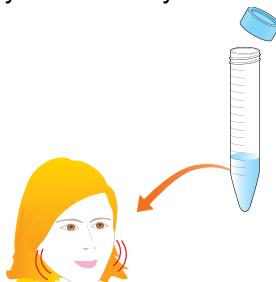
##### Steps 1 and 2: Collecting and breaking open cells

To collect as many cheek cells as possible, you will gently chew the insides of your mouth for 30 seconds and then rinse your mouth with a small amount of water. Ample cell collection is critical for success. For best results, make sure you spend the recommended amount of time collecting the cells.

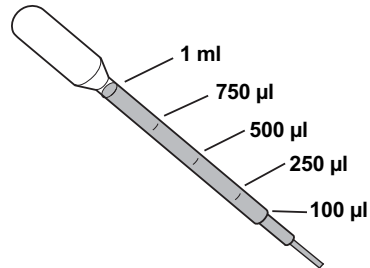
1. Obtain a 15 ml tube containing a 3 ml of water, and label it with your initials.



2. Gently chew the insides of your mouth for 30 seconds.
3. Take the 3 ml of water from your tube into your mouth and rinse vigorously for 30 seconds. Don't swallow the water!



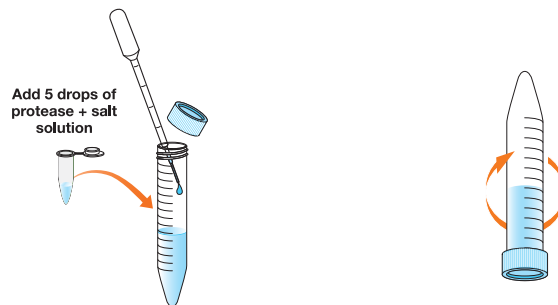
- Carefully expel all your water mouthwash back into your 15 ml tube.
- Locate the 15 ml tube at your workstation labeled '**lysis**'. Using a fresh disposable plastic transfer pipet, add 2 ml of lysis buffer to your tube.



- Place the cap back in your tube. Gently invert your tube 5 times to lyse your cells. Don't shake the tube. If you observe any changes to your cells at this time, write them down.

### Step 3: Removing proteins

- Obtain the pink microcentrifuge tube labeled '**prot**' and add 5 drops of protease and salt solution to the 15 ml tube containing your cell extract. Cap the cell extract tube and gently invert it 5 times to mix.



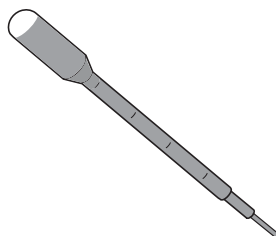
- Place your cell extract tube in the beaker or test tube holder in the 50°C water bath (at the common workstation) for 10 minutes to allow the protease to work.



50°C for 10 min

### Steps 4 and 5: Making the DNA visible

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

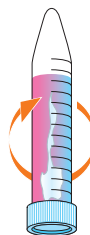




- Tilt your 15 ml tube at a 45° angle and slowly add the alcohol, carefully letting it flow gently down the inside of the tube. Fill the tube with cold alcohol (about 10 ml total). You may need to use several pipets full of cold alcohol. You should be able to see two layers (upper and lower) forming. As you add the alcohol, pay close attention to the place where the alcohol and cell extract layers meet. Write down your observations.

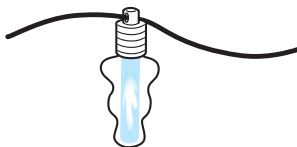


- Place your 15 ml tube upright either on the cup or a test tube and **leave it undisturbed** at room temperature for 5 minutes.
- After 5 minutes, look again at the contents of your tube, especially in the area where the alcohol and cell extract layers meet. Do you see anything? Write down your observations. Compare your sample with those of your classmates.
- With the cap of your tube tightly sealed, mix the contents of your tube by slowly inverting the tube 5 times. Look for any stringy, white or clear material. **This is your DNA!**

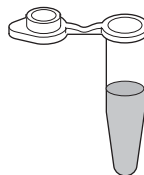


- If you are going to make a DNA necklace, your teacher will provide you with a helix vial, screw cap, and cord. With a disposable transfer pipet, carefully transfer the precipitated DNA along with approximately 500  $\mu$ l of the alcohol solution into the vial, screw on the cap tightly.

If you are not going to make a DNA necklace, you can transfer and save your DNA in a 1.5 ml microcentrifuge tube. With a disposable plastic transfer pipet, gently withdraw your precipitated DNA along with about 1 ml of alcohol solution and transfer it into the microcentrifuge tube. Tighten the cap and amaze your friends and family with your own DNA!



or



## 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

Fast Blast™ DNA stain can be used as a nuclear stain for cheek cells. The positively charged dye molecules of Fast Blast bind to the negatively charged phosphate groups on DNA. Using the same stain to visualize DNA in agarose gels as well as in cell nuclei can help your students understand where DNA resides in eukaryotic cells.

**Note:** Fast Blast stain is not toxic but it will stain skin and clothing. Wear gloves and a lab coat whenever handling the stain solutions.

### Teacher's Advance Preparation

#### Objective

Prepare 1:50 dilution of Fast Blast stain (and 1x PBS, if necessary)\*

#### Required Materials

- 500x Fast Blast DNA stain (catalog #166-0420EDU)
- 200 µl micropipet or disposable plastic pipet tips
- Micropipet tips (if using a micropipet)
- 5 ml isotonic saline solution (e.g., contact lens saline solution or 1x PBS)\*

#### Procedure

Dilute the 500x Fast Blast DNA stain before using it to visualize the nuclei of cheek cells. To prepare 5 ml of diluted stain, add 100 µl of 500x Fast Blast to 4.9 ml of isotonic saline solution.

**Note:** An isotonic solution is necessary to maintain the proper osmotic balance across the cell membrane.

\* 10x PBS can be purchased (#166-2403EDU). Dilute to 1x using distilled water. Alternatively, prepare 1x PBS by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na<sub>2</sub>HPO<sub>4</sub>, and 0.24 g of KH<sub>2</sub>PO<sub>4</sub> in 800 ml of distilled water. Adjust the pH to 7.4 with HCl or NaOH and then bring the volume to 1 L with distilled water. Sterilize by autoclaving or by filtering, and store at room temperature.

## Required Equipment and Accessories

- Microscope
- Microscope slides
- Glass coverslips
- Disposable plastic transfer pipet or dropper
- Sterile micropipet tips\*
- 20  $\mu$ l micropipet

## Procedure

### Collect cheek cells

Collect cheek cells by gently scraping the inside of your cheeks 10 times with a sterile pipet tip.\* This is most easily done by pinching and extending the corner of your mouth with one hand, and scraping the cheek with the tip in the other hand. Use firm but gentle pressure. You should see a volume of white cells at the end of the pipet tip.

### Stain cells

1. Add a drop of diluted Fast Blast to the microscope slide. Place the pipet tip with the cheek cells on the end of the a micropipet that is set to 20  $\mu$ l. Gently pipet up and down 5 times to transfer your cheek cells into the drop of Fast Blast stain.
2. Cover the sample with a clean coverslip. View the slide under a microscope at low and medium power magnifications.

**Note:** Students should have had prior instruction in the proper use of a microscope. Fast Blast stains cell nuclei within 2 minutes. Have your students make observations and draw sketches, labeling visible cell structures.

\* If a micropipet and tip are not available, a cytology brush or cotton swab can be used to collect and mix the cells into the diluted Fast Blast stain.

## Staining precipitated DNA Using Concentrated Fast Blast DNA Stain (Bio-Rad catalog #166-0420EDU)

1. Using a disposable transfer pipet transfer your precipitated DNA (from step 13 in the Quick Guide on page 12) and less than 1 ml of alcohol from the 15 ml tube to a 1.5 ml microcentrifuge tube. Using the same disposable transfer pipet, remove excess alcohol, leaving approximately 750  $\mu$ l in your tube.
2. Add 500  $\mu$ l of 500x Fast Blast DNA stain to the microcentrifuge tube, and let the DNA stain for at least 10 minutes.
3. Using a disposable plastic transfer pipet, transfer all the liquid, including the DNA, from the microcentrifuge tube to a 15 ml tube containing 10 ml of 70% alcohol. Let sit for 5 minutes.
4. Pipet or decant off as much of the alcohol as possible. Take care not to lose your DNA! Fill the 15 ml tube to the 10 ml mark with fresh 70% alcohol. Let sit for 5 minutes.
5. (Optional) Continue with step 13 of the Quick Guide (page 12 in the manual) to save your stained DNA in a micro test tube, or create a DNA necklace.

## **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.

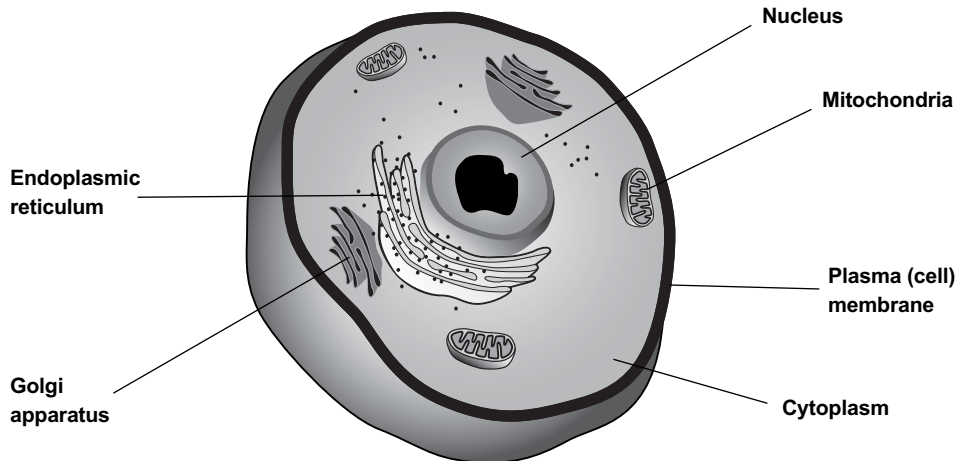
2. **Does a liver cell contain the same chromosomes as a cheek cell?**

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.**

   A Harvest the cells

   C Dissolve cell membranes

   D Precipitate the DNA

   B Break down proteins

   E Make DNA less soluble in water

A. Gently chew the insides of your mouth and then rinse vigorously with water

B. Add protease, incubate at 50°C

C. Mix in a detergent solution

D. Layer cold alcohol over cell extract

E. Add salt





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