



The Biotechnology Education Company ®

EDVO-Kit #

**119**

## **Genes in a Tube™**

Storage: See Page 3 for  
specific storage instructions

### **EXPERIMENT OBJECTIVE:**

The purpose of the experiment is to rapidly extract "self"  
DNA from cheek cells, to visualize DNA, and to store DNA  
in a Genes in a Tube™ necklace.

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Safety Data Sheets can be found on our website:  
[www.edvotek.com/safety-data-sheets](http://www.edvotek.com/safety-data-sheets)

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.



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## Experiment Components

This experiment is  
for 26 DNA isolations.

Store entire experiment at  
room temperature.

- Lysis Buffer
- NaCl Solution
- Protease
- Tris Buffer
- Flash Blue™ solution
- Salt packets

### Storage

Room temperature  
Room temperature  
Room temperature  
Room temperature  
Room temperature  
Room temperature

- 
- Clear tubes for DNA isolation
  - Microcentrifuge tubes with caps
  - Sterile cotton tipped applicators
  - Small transfer pipets
  - Calibrated transfer pipets
  - String for Genes in a Tube™ necklaces
  - Disposable plastic cup

## Requirements

- Freezer/ice cold 95% Ethanol or isopropyl alcohol (rubbing alcohol)
- Water bath
- Test tube racks
- Ice and ice buckets
- Disposable laboratory gloves
- Centrifuge

## Background Information

### AN INTRODUCTION TO DNA

All living organisms are composed of cells. Organisms such as bacteria are single cells, while very complex organisms, such as humans, are composed of billions of many different cells. A biologist named Friedrich Miescher carried out research in 1868 which indicated that the nucleus of cells contains a material which he called nucleic acid. It was not until much later in the 1940's that deoxyribonucleic acid (DNA), was recognized as the carrier of the genetic code.

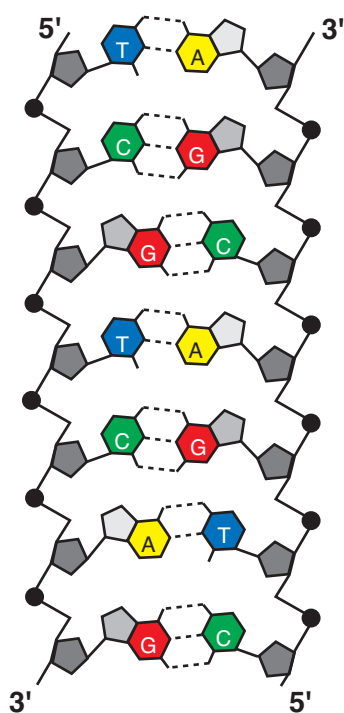


Figure 1

The DNA structure was determined by James Watson and Francis Crick in 1953. They determined that DNA was a double helix consisting of two strands with opposite polarity. The Watson and Crick model is often described as the spiral DNA ladder.

Human cells contain a nucleus, which contains 46 chromosomes (23 pairs). Chromosomes contain DNA which encodes all the genetic information that is inherited from the two biological parents. DNA is made up of building blocks known as nucleotides. Each nucleotide is composed of three parts, a phosphate group, deoxyribose sugar, and one of the four nitrogenous bases, Adenine, Guanine, Cytosine, or Thymine.

The two strands of DNA that make up the backbone of the DNA ladder is made up of the deoxyribose carbohydrate units linked together by phosphodiester bonds. The carbohydrate backbone acts as a support for the rungs of the ladder. The rungs are composed of the nitrogenous bases. The first letters of these bases, A, G, C, and T, designate the order of the bases within the two DNA strands. The four bases are always hydrogen bonded in pairs. When A occurs on one strand, T will occur on the opposite strand. Similarly, G and C are base pairs on opposite DNA strands. The bases are held together by hydrogen bonds which are shown as dashed lines in Figure 1. Unlike covalent bonds hydrogen bonds are much weaker.

## Background Information

The human genome consists of 2.9 billion base pairs. Of this total, only about 5% code for protein. Intervening sequences and other noncoding sequences make up the remainder. Some of the noncoding sequences or other functionally unassigned sequences may possess undiscovered functions. Certain noncoding sequences appear to be self-replicating and are repeated hundreds or thousands of times throughout the genome. These repetitive sequences have been termed “selfish” or “parasitic” DNA, as they often appear to possess no function except that of their own reproduction. Repetitive elements account for more than 20 percent of the human genome. In addition to genomic DNA, mitochondria, which are cellular organelles, contain their own DNA (mitochondrial DNA) which replicate independently from cell chromosomal DNA.

During the process of replication, DNA provides the required information to copy (replicate) itself, which results in genetic information to be passed on to the next generation of cells. DNA also provides the instructions for making proteins for various cell functions. A large number of proteins and enzymes including DNA polymerases are involved in the synthesis of DNA. In addition to synthesis DNA polymerases have the ability to edit newly synthesized DNA for possible errors in base pair matching and to participate in repairing DNA that gets damaged during the life of the cell. Such damage to DNA can occur due to exposure to carcinogens and environmental factors such as X-rays and U.V. light from exposure to the sun.

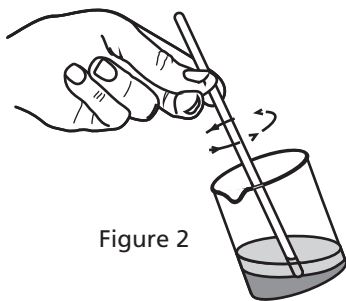


Figure 2

When cells are chemically lysed (broken open), DNA from chromosomes is released and can be isolated and purified. DNA extraction is frequently the first step for molecular biology and biotechnology experiments. Extracted DNA is soluble in water and thus will appear as a clear solution. By contrast DNA is insoluble in salt solutions and alcohol, where it will form white fibers.

Purification procedures for DNA usually include precipitation with alcohol in the presence of salt. The DNA solution is carefully overlaid with alcohol. Since alcohols such as rubbing alcohol (isopropyl alcohol) have lower density than water a second layer above the DNA solution will be formed. A glass rod or a stirrer is used to spool DNA at the interface of the two liquid phases and to separate the DNA from the solution (see Figure 2). The DNA will appear as a viscous, clotted mass, which can be collected on a stirrer or a glass rod. The amount of DNA spooled will vary and is a consequence of the intactness of the DNA sample.

## Background Information

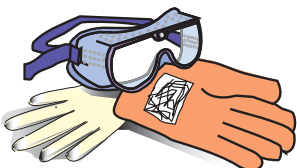
Almost any tissue or body fluid (except urine) may be used as a source of DNA. The most common sources of human DNA are samples from hair, cheek cells, blood and saliva. Once extracted, DNA can be stored for long periods of time. Various methods of storage include precipitation and storage under alcohol at room temperature or under refrigeration. Such amounts of DNA can be obtained from individuals during medical procedures or evidence left behind in crime scenes such as cells that are recovered from the fingernails of a victim. In recent times, a few cells deposited by a person while licking and sealing an envelope has been sufficient to obtain DNA and match the DNA fingerprint to the person who left this evidence behind.

A specified amount of DNA is required for DNA fingerprinting. Small amounts of body fluids or tissues until recently was not sufficient for DNA extraction and analysis. This is no longer a limitation if genes to be analyzed are first amplified by the polymerase chain reaction (PCR) prior to use. In 1984 Kary Mullis invented PCR and for his unique contribution he was awarded a Nobel Prize in 1994. The widespread utility of PCR is based on ease of use and the ability to amplify DNA making it possible to perform a variety of studies including DNA fingerprinting. DNA amplified by the PCR reaction is analyzed by agarose gel electrophoresis or become part of additional procedures used in DNA studies.

In this experiment individual "self" DNA will be extracted from cheek cells and stored in a "Genes in a Tube™" necklace. DNA isolated from this experiment can be recovered and used for DNA fingerprinting and analysis of an individual's genetics.



## Experiment Overview and General Instructions



Wear gloves  
and goggles

### EXPERIMENT OBJECTIVE:

The purpose of the experiment is to rapidly extract "self" DNA from cheek cells, to visualize DNA, and to store DNA in a "Genes in a tube™" necklace.

### LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Properly dispose materials after completing the experiment:
  - A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.
  - B. All materials, including cups, tubes, and pipets should be disinfected before disposal in the garbage. Soak in 10% bleach solution. Immerse open tubes and other materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.




### Experiment Overview - Option A DNA Isolation from Cheek Cells Using A Cotton Swab

#### Experiment Procedure

**1**

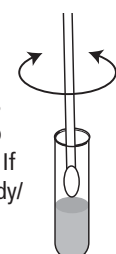
Extract DNA

Swab cheek cells vigorously



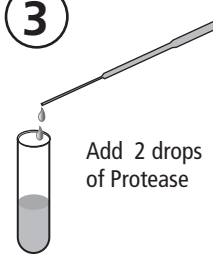
**2**

Twirl applicator in lysis buffer to release cells. Press swab against side of tube to release all the liquid (cells). If buffer does not appear cloudy/turbid, repeat step 1 with a new swab.



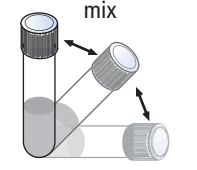
**3**

Add 2 drops of Protease



**4**

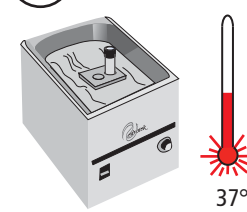
Cap tube and Invert to mix



**5**

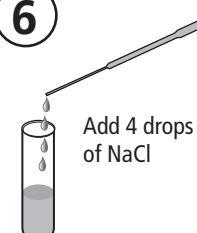
incubate 10-15 min.

37°C



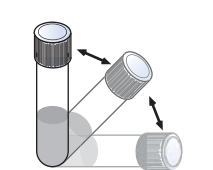
**6**

Add 4 drops of NaCl



**7**

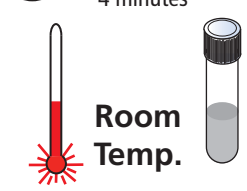
Cap tube and Invert to mix



**8**

Incubate for 4 minutes

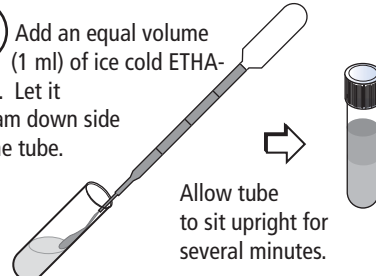
Room Temp.



**9**


Add an equal volume (1 ml) of ice cold ETHA-NOL. Let it stream down side of the tube.

Allow tube to sit upright for several minutes.



**10**

Look for DNA (stringy/white material) at the interface.

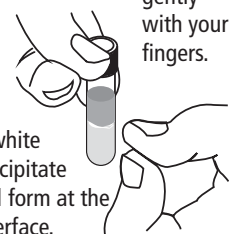


**11**

Flick tube gently with your fingers.


A white precipitate will form at the interface.

This is your DNA!!




**12**

Transfer a small amount of DNA to the tube for the "Genes in a Tube" necklace.




**14**

Transfer some of the stained DNA to the necklace tube.




**13**

Add a small drop (half of the tip of a small transfer pipet) of Flash Blue to the tube. Flash Blue will dye the DNA precipitate dark blue.



**15**

"Genes in a Tube" with unstained & stained DNA.





## Option A: DNA Isolation from Cheek Cells Using A Cotton Swab

This experiment can alternatively be performed using a saline solution rinse alone (see pages 11-13). There is enough material to perform either Option A or Option B of the experiment.

## Optional saline rinse.

Adding a saline rinse will help loosen up cheek cells in the mouth and make the swabbing process yield more cells.

To do this, obtain a cup containing 3 ml of saline solution from your instructor. Put the saline solution into your mouth and swirl it around vigorously for 45 seconds.

## OPTIONAL STEP

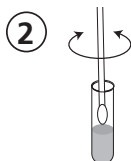
Before collecting cheek cells with a cotton swab



Rinse mouth with saline rinse



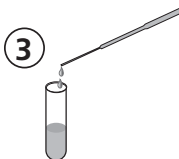
1. Isolate DNA from your cheek cells. Using a sterile cotton-tipped applicator, collect cheek cells by vigorously swabbing inside the mouth along the gum line and under the tongue.
2. Place the applicator in the tube containing Lysis buffer and twirl to dislodge the cells. Press the tip to the sides of the tube to squeeze out as much liquid as possible.



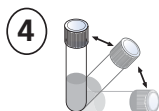
Repeat steps 1 and 2 using a fresh sterile applicator if buffer is clear. Twirl the applicator in the same tube containing lysis buffer to add cells to the solution.

**Important Note:**

**Cheek cells in buffer must appear cloudy/turbid.**



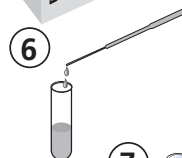
3. Using a small transfer pipet, add 2 drops of Protease solution to the tube.



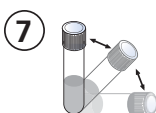
4. Cap and gently invert the tube several times to mix.



5. Incubate the tube in 37°C waterbath for 10-15 minutes. (The protease is most active at 37°C.)



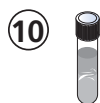
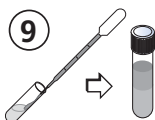
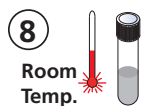
6. Using a new transfer pipet, add 4 drops of NaCl solution to the tube.



7. Cap the tube and mix the contents by inverting the tube (or gently flick it with your index finger) several times.

## Option A: DNA Isolation from Cheek Cells Using A Cotton Swab

## Experiment Procedure



Flash blue is a DNA stain which will make the extracted DNA more easily visible to the naked eye. Your DNA will appear as heavy blue gel-like strands at the alcohol and aqueous interface. The solution will also have a light blue color but will be lighter than your DNA.

8. Incubate the tube at room temperature for 4 minutes.

9. Precipitate the DNA from solution.

- Using a clean calibrated transfer pipet, add an equal volume (1 ml) of ice cold alcohol to the test tube. Hold the test tube at a 45° angle and gently pipet the cold alcohol allowing it to flow down the side of the tube.
- Place the tube in a test tube rack sitting upright. Allow the tube to sit for 3-5 minutes.

10. Check that your DNA is visible at the interface of the alcohol/salt solution. Some DNA may appear as stringy material, grayish-white in color.

11. Cap the tube and gently flick the tube several times with your fingers. Alternatively, you can invert the tube once to disperse the DNA throughout the solution. A precipitate (stringy white or clear material) will form at the interface. This is your DNA!

12. Transfer a small amount of DNA to the Genes in a Tube™ necklace.



13. Stain the remaining precipitated DNA. Use a microtipped transfer pipet to add a small drop (half of the tip of the transfer pipet) of Flash Blue™ solution to the tube. Squeeze the neck of the transfer pipet, not the bulb.



14. Transfer some of the stained DNA to the tube for the necklace.



15. Your tube for the "Genes in a Tube" necklace now contains both unstained and stained DNA.

16. After removing the portions of your unstained and stained DNA for transfer to the necklace tube, recap the tube containing your precipitated DNA. Label the tube with your initials.

17. Place the DNA in the freezer and observe the DNA in the tube the next day or at a later time. the transfer pipet, not the bulb.

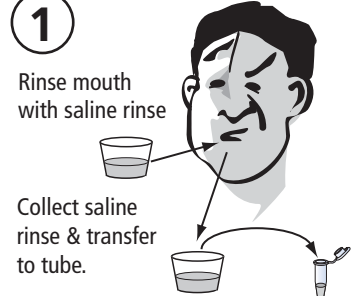
18. Properly dispose materials after completing the experiment. (See Laboratory Safety, page 7.)

### Experiment Overview - Option B

#### DNA Isolation from Cheek Cells Using A Saline Solution Rinse

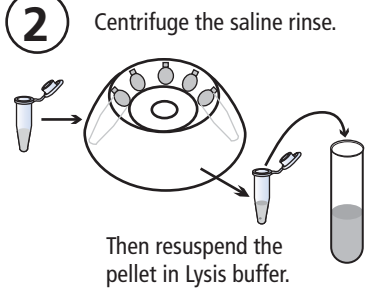
**1** Rinse mouth with saline rinse

Collect saline rinse & transfer to tube.

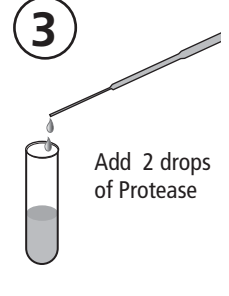


**2** Centrifuge the saline rinse.

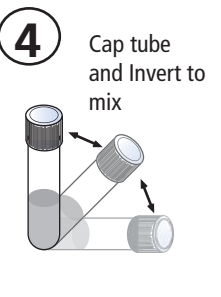
Then resuspend the pellet in lysis buffer.



**3** Add 2 drops of Protease

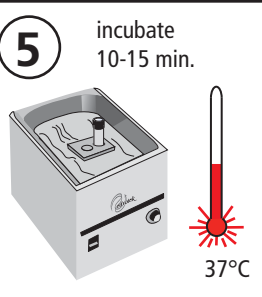


**4** Cap tube and invert to mix

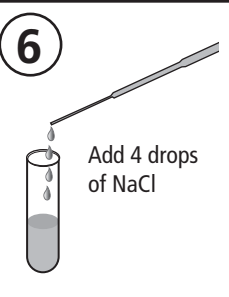


**5** incubate 10-15 min.

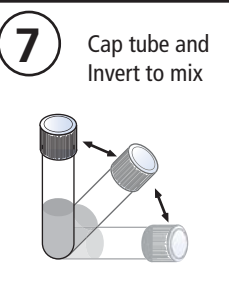
37°C



**6** Add 4 drops of NaCl

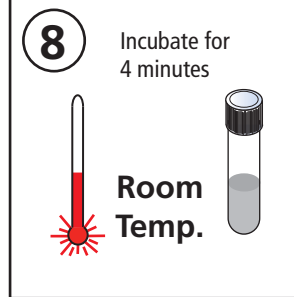


**7** Cap tube and invert to mix



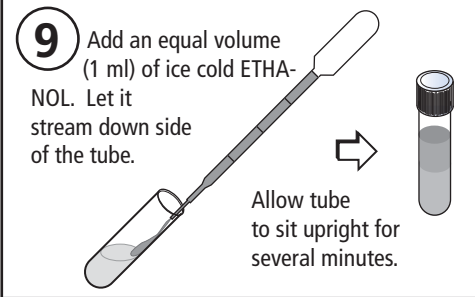
**8** Incubate for 4 minutes

Room Temp.

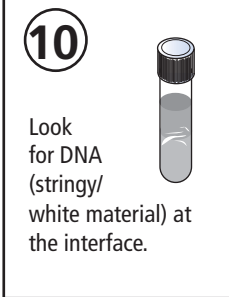


**9** Add an equal volume (1 ml) of ice cold ETHA-NOL. Let it stream down side of the tube.

Allow tube to sit upright for several minutes.



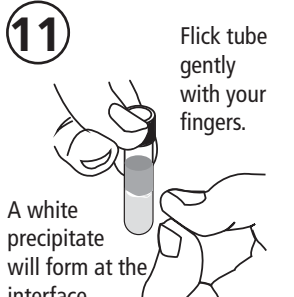
**10** Look for DNA (stringy/white material) at the interface.



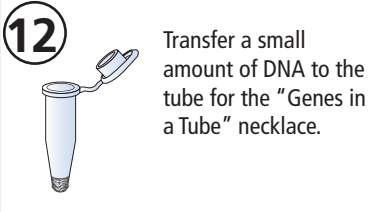
**11** Flick tube gently with your fingers.

A white precipitate will form at the interface.

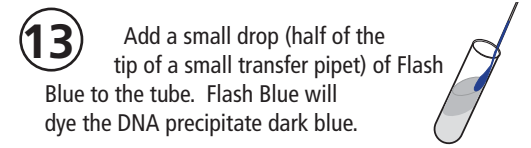
This is your DNA!!



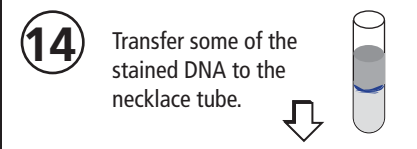
**12** Transfer a small amount of DNA to the tube for the "Genes in a Tube" necklace.



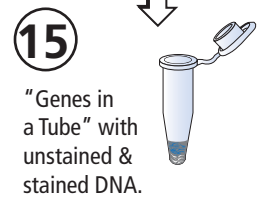
**13** Add a small drop (half of the tip of a small transfer pipet) of Flash Blue to the tube. Flash Blue will dye the DNA precipitate dark blue.



**14** Transfer some of the stained DNA to the necklace tube.




**15** "Genes in a Tube" with unstained & stained DNA.



Experiment Procedure

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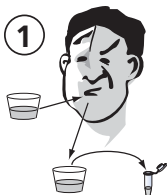
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11

## Option B: DNA Isolation from Cheek Cells Using A Saline Solution Rinse

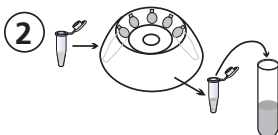
This experiment can alternatively be performed using a cotton swab (see pages 8-10). There is enough material to perform either Option A or Option B of the experiment.

### Experiment Procedure



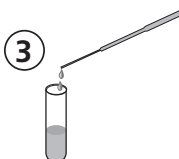
#### 1. Isolate DNA from your cheek cells.

- Obtain a cup containing 3 ml saline solution from your instructor. Clearly label the cup with your initials or name.
- Put the saline solution into your mouth and swirl it around vigorously for 45 seconds.
- Carefully return the saline rinse containing the harvested cheek cells to the cup.

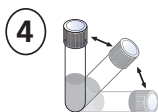


#### 2. Centrifuge the collected rinse solution containing your cheek cells and resuspend the pellet in lysis buffer.

- Label a 2.0 ml test tube with your initials and transfer 1.5 ml of your saline rinse into this tube.
- Spin the tube in a microcentrifuge at maximum speed for 2 minutes.
- Check for the presence of a white pellet at the bottom of the tube after centrifugation.
- Carefully remove and discard the supernatant.
- Then refill your tube with an additional 1.5 ml of your saline rinse, and repeat the spin.
- Obtain a clean capped tube containing 1 ml of lysis buffer. Write your initials on the side of the tube with a lab marker.
- Remove and discard the supernatant. To the remaining cell pellet, add 200  $\mu$ l of lysis buffer. Resuspend the pellet by pipetting or vortexing.
- Transfer the entire content of the microtest tube in step 6 to the tube containing the remaining 800  $\mu$ l of lysis buffer. Cap and mix gently.



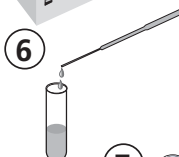
#### 3. Using a small transfer pipet, add 2 drops of Protease solution to the tube.



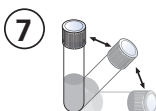
#### 4. Cap and gently invert the tube several times to mix.



#### 5. Incubate the tube in 37°C waterbath for 10-15 minutes. (The protease is most active at 37°C.)

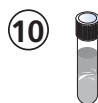
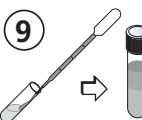
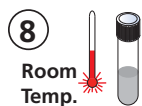


#### 6. Using a new transfer pipet, add 4 drops of NaCl solution to the tube.



#### 7. Cap the tube and mix the contents by inverting the tube (or gently flick it with your index finger) several times.

## Option B: DNA Isolation from Cheek Cells Using A Saline Solution Rinse



Flash blue is a DNA stain which will make the extracted DNA more easily visible to the naked eye. Your DNA will appear as heavy blue gel-like strands at the alcohol and aqueous interface. The solution will also have a light blue color but will be lighter than your DNA.

8. Incubate the tube at room temperature for 4 minutes.

9. Precipitate the DNA from solution.

- Using a clean calibrated transfer pipet, add an equal volume (1 ml) of ice cold alcohol to the test tube. Hold the test tube at a 45° angle and gently pipet the cold alcohol allowing it to flow down the side of the tube.
- Place the tube in a test tube rack sitting upright. Allow the tube to sit for 3-5 minutes.

10. Check that your DNA is visible at the interface of the alcohol/salt solution. Some DNA may appear as stringy material, grayish-white in color.

11. Cap the tube and gently flick the tube several times with your fingers. Alternatively, you can invert the tube once to disperse the DNA throughout the solution. A precipitate (stringy white or clear material) will form at the interface. This is your DNA!

12. Transfer a small amount of DNA to the Genes in a Tube™ necklace.



13. Stain the remaining precipitated DNA. Use a microtipped transfer pipet to add a small drop (half of the tip of the transfer pipet) of Flash Blue™ solution to the tube. Squeeze the neck of the transfer pipet, not the bulb.



14. Transfer some of the stained DNA to the tube for the necklace.



15. Your tube for the "Genes in a Tube" necklace now contains both unstained and stained DNA.

16. After removing the portions of your unstained and stained DNA for transfer to the necklace tube, recap the tube containing your precipitated DNA. Label the tube with your initials.

17. Place the DNA in the freezer and observe the DNA in the tube the next day or at a later time. the transfer pipet, not the bulb.

18. Properly dispose materials after completing the experiment. (See Laboratory Safety, page 7.)

### Study Questions

1. Describe the appearance of the isolated DNA.
2. What do cell nuclei contain?
3. What are nucleotides?
4. How many chromosomes do humans have?
5. What does cell lysis mean?
6. Why did the rubbing alcohol form a layer on top of the DNA solution?

## Instructor's Guide

### Notes to the Instructor & Pre-Lab Preparations

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in planning and implementing this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

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### Pre-Lab Preparations for Option A: DNA Isolation from Cheek Cells Using A Cotton Swab

1. Add 200 µl of Tris buffer to the tube of protease. Allow the material to hydrate for a few minutes and transfer the entire amount back to the remaining Tris buffer.

Mix well and aliquot 100 µl for each student pair. Be sure to place the tubes on ice until they are needed.

2. Aliquot 3 ml of Ethanol or Isopropyl alcohol for each student pair. Place on ice until needed.
3. Aliquot 200 µl of NaCl solution for each student pair.
4. Aliquot 200 µl of Flash Blue solution for each student pair.
5. Aliquot 1 ml of Lysis Buffer into clear tubes (provided) - one per student.
6. Optional Step: Dissolve all 8 salt packets in 500 ml of drinking water to make the saline solution. Aliquot 3 ml per cup per student.

#### Reagents to be Shared by Two Students:

- 100 µl Protease solution
- 200 µl NaCl solution
- 200 µl Flash Blue solution
- 3 ml Ice cold alcohol (95% ethanol or Isopropyl rubbing alcohol)

#### Each Student should receive:

- 1 ml Lysis buffer in clear tubes with caps
- 1 Package of two sterile cotton-tipped applicators
- 3 Small transfer pipets
- 1 Calibrated transfer pipet
- 1 Tube & string to make the Genes in a Tube necklace.
- 1 Cup of saline solution (optional)



**Pre-Lab Preparations for Option B:  
DNA Isolation from Cheek Cells Using A Saline Solution Rinse**

1. Add 200 µl of Tris buffer to the tube of protease. Allow the material to hydrate for a few minutes and transfer the entire amount back to the remaining Tris buffer.  
  
Mix well and aliquot 100 µl for each student pair. Be sure to place the tubes on ice until they are needed.
2. Aliquot 3 ml of Ethanol or Isopropyl alcohol for each student pair. Place on ice until needed.
3. Aliquot 200 µl of NaCl solution for each student pair.
4. Aliquot 200 µl of Flash Blue solution for each student pair.
5. Aliquot 1 ml of Lysis Buffer into clear tubes (provided) - one per student.
6. Dissolve all 8 salt packets in 500 ml of drinking water to make the saline solution. Aliquot 3 ml per cup per student.

**Reagents to be Shared by Two Students:**

- 100 µl Protease solution
- 200 µl NaCl solution
- 200 µl Flash Blue solution
- 3 ml Ice cold alcohol (95% ethanol or Isopropyl rubbing alcohol)

**Each Student should receive:**

- 1 ml Lysis buffer in clear tubes with caps
- 1 microcentrifuge tube (2.0 ml)
- 3 Small transfer pipets
- 1 Calibrated transfer pipet
- 1 Tube & string to make the Genes in a Tube necklace.
- 1 Cup of saline solution

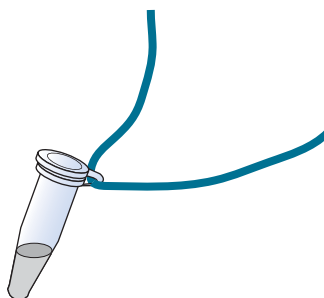
## Inquiry-based Optional Extension Experiments

Encourage students to think of different parameters in the experiment to alter and have them predict the outcome. Listed below are several examples of optional activities for students to try.

- Perform an experimental control with just the lysis buffer (cheek cells are not added). Add the protease and NaCl and overlay the solution with alcohol. Note the differences between this control tube and the sample tubes containing DNA.
- Perform the DNA isolation and eliminate the protease, NaCl, and/or alcohol from the isolation steps. What happens to the DNA sample?
- After the addition of alcohol, isolate the DNA by spooling. DNA can be recovered by dissolving in distilled water overnight and then prepared for agarose gel electrophoresis (materials for electrophoresis not included).
- Isolate the DNA and perform PCR on the DNA sample (materials for PCR not included).

## Expected Results

Genes in a Tube™



## Study Questions and Answers

## 1. Describe the appearance of the isolated DNA.

DNA looks like a clear liquid gel. It turns white when it forms a precipitate.

## 2. What do cell nuclei contain?

The nucleus is an organelle present in human cells. It contains the chromosomes that are rich in DNA.

## 3. What are nucleotides?

Nucleotides are the building blocks of DNA.

## 4. How many chromosomes do humans have?

There are 46 chromosomes (23 pairs) in human cells.

## 5. What does cell lysis mean?

Cell lysis is the disruption of a cell and cell nucleus with the release of the contents.

## 6. Why did the rubbing alcohol form a layer on top of the DNA solution?

Alcohol has lower density than water and therefore will "float" above the DNA solution.