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INDIANA UPLANDS | PROUD PARTNER

Grade 6-8 STEM Challenge

Cheek Cells & DNA

Inspired by Dathan, a Biomedical Manufacturing Operator in the Indiana Uplands.

Published by Regional Opportunity Initiatives

GRADE 6-8 STEM CHALLENGE

Cheek Cells & DNA

Inspired by Dathan, a Biomedical Manufacturing Operator in the Indiana Uplands.

Students will examine their own cheek cells and extract DNA using a common method called ethanol precipitation.

CAREER CONNECTION AND LESSON OVERVIEW

Dathan is a biomedical manufacturing operator at Catalent in Bloomington, Indiana. He works in a lab where he grows cell cultures that are modified to produce vaccines and medications. Once there are enough cells to move into production, Dathan isolates the needed proteins, performs testing and collects data to make sure that the substance will be pure enough to be approved for further testing and use.

To get the cells to make the proteins that he wants, Dathan has to give them new instructions. The scientists and technicians at Catalent insert new DNA with the gene of interest into the cells, let those cells grow, and harvest the new proteins they make. In this activity, students will examine their own cheek cells and then extract DNA from them.

LESSON TIMELINE

DAY 1
- Show the inspiration video, "Dathan - Drug Substance Operator" (10 minutes)
- Introduce the protocol (5 minutes)
- Prepare and examine a wet-mount slide of stained cheek cells

DAY 2
- Reframe the experiement (10 minutes)
- Student DNA extraction (35 minutes)

DAY 3
- Complete DNA extraction (if not finished on Day 2)
- Finish data sheet and report out (30 minutes)

Recommended Supplies

For Cheek Cell Slides:
- Sterile cotton swabs or flat toothpicks
- Clean microscope slides
- Coverslips
- Methylene blue solution (0.5-1%)
- DropperBlotting paper/paper towels
- Microscope

For DNA Extraction (2 students per group)
- Two 15ml test tubes, with sealable cap
- Two 1.5ml Eppendorf tubes or similar
- 0.9% salt water (9g NaCl in 1L of water)
- Meat tenderizer, unseasoned. This may be added dry (tiny pinch per tube) or mixed 1 part powder with 19 parts water
- 2 small disposable cups
- 25% solution of dish soap
- 10 ml 90+% isopropyl alcohol OR 90+% ethanol, chilled overnight in the freezer
- 2 bamboo skewers
- Plastic droppers or pipettes

IN THIS CHALLENGE, STUDENTS WILL:

- Prepare and observe a wet-mount slide of their cheek cells stained with methylene blue and identify the nucleus.
- Conduct an alcohol precipitation reaction to isolate their own DNA from their cheek cells.

Standards

Science & Engineering Process Standards
SEPS.1 Posing Questions (for science) and defining problems (for engineering)
SEPS.2 Developing and using models and tools
SEPS.4 Analyzing and interpreting data
SEPS.6 Constructing explanations (for science) and designing solutions (for engineering)
SEPS.8 Obtaining, evaluating, and communicating information

Preparing for College and Careers
PCC-2.1 Determine roles, functions, education, and training requirements of various career options within one or more career clusters and pathways
PCC-2.2 Analyze career trends, options and opportunities for employment and entrepreneurial endeavors for selected career clusters and pathways
PCC-2.3 Evaluate selected careers and pathways for education requirements, working conditions, benefits, and opportunities for growth and change
PCC-2.4 Use appropriate technology and resources to research and organize information about careers

7th Grade Science Standards
7.LS.1 Investigate and observe cells in living organisms and collect evidence showing that living things are made of cells. Compare and provide examples of prokaryotic and eukaryotic organisms. Identify the characteristics of living things.

8th Grade Science Standards
8.LS.6 Create models to show how the structures of chromatin, chromosomes, chromatids, genes, alleles and deoxyribonucleic acid (DNA) molecules are related and differ
8.LS.10 Gather and synthesize information about how humans alter organisms genetically through a variety of methods

Grade 6-8 Employability Skills
6-8.M.1 Apply new strategies based on lessons learned from feedback
6-8.WE.3 Complete tasks or activities with some prompting and guidance
6-8.LS.4 Identify possible career choices and high school course selection using self-assessment (including an appraisal of strengths, interests, and values)
6-8.LS.7 Evaluate decisions and discuss the use of alternatives in decision-making situations.
6-8.LS.12 Use prediction and evaluation skills to develop potential solutions

Planning and Implementation

CHEEK CELLS & DNA

Essential Vocabulary

- CELLS: The smallest unit of an organism that can be called alive, typically microscopic and consisting of cytoplasm and DNA.
- NUCLEUS: A membrane-bound organelle within the cells where DNA is kept.
- DNA: An acronym for deoxyribonucleic acid, a self-replicating molecule present in nearly all living organisms and makes up chromosomes. It is the carrier of genetic information.
- CHROMOSOMES: Long, organized chains of DNA
- PRECIPITATION: The condensation of a solid product from a liquid solution.
- ENZYME: A protein that facilitates a specific chemical reaction, usually a catalyst.
- PROTEASE: An enzyme that breaks down proteins.
- LYSIS: The act of breaking open cells to get to the contents

In this challenge, students will:

- Prepare and observe a wet-mount slide of their cheek cells stained with methylene blue and identify the nucleus.
- Conduct an alcohol precipitation reaction to isolate their own DNA from their cheek cells.

Before Class:

- Read the activity outline sheet and leader notes to become familiar with the activity.
- Gather necessary materials. Be sure that you have enough materials and space for each student to create their own slides and isolate their own DNA. Note: do NOT have students isolate each other's DNA.
- Students should have an understanding of how to use a microscope and prepare a slide before beginning this experiment.
- Students should understand basic lab safety.

Guiding Questions

1. What is DNA?
2. What is a nucleus?
3. How does DNA store information?
4. How do scientists change other organisms to make products needed to treat human diseases?

Day 1

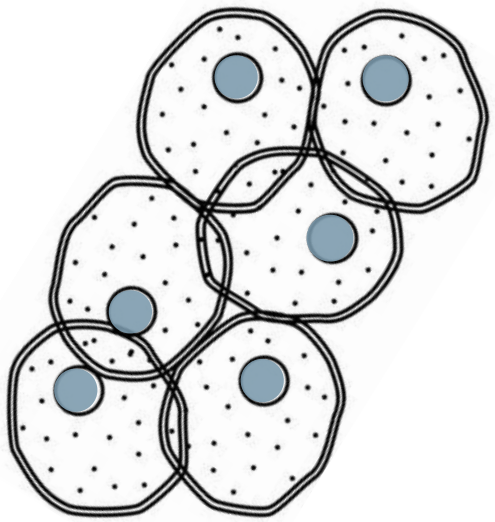
Introduction

Show students Dathan's career shadow video. Dathan works at Catalent, a pharmaceutical company that transforms cells to make them manufacture needed products—usually proteins. Cells and the DNA within them are critical to the manufacturing of these proteins. In this series of activities, students will examine stained cheek cells and the isolate tnheir own DNA.

Provide each student with a protocol sheet. This is an excellent activity to encourage good science notebooking habits and get students thinking like real scientists. If your students have never used light microscopes, ensure that they are familiar with their basic use and safety. Alternatively, students can create their cheek cell slides and examine them under a microscope connected to a projection system. This can also be done by the instructor as a demonstration, but it is critical that students make the connection between the cellular structures they observe and the DNA they isolate in part 2.

Part 1: Examining Cheek Cells

Students have probably heard of and/or seen pictures of cells before but it isn't quite the same as getting to see your own cells! Today students will create a wet-mount slide of their own cheek cells and identify the nucleus. We will be staining the cells with methylene blue, a common cellular dye that will turn the nucleus of the cells blue by sticking to the DNA. Once stained, students will be able to identify some of the organelles within the cells using a light microscope.



Note: This activity should be done after learning how to use a light microscope. Alternatively, this can be done as a demonstration and shared with the class using a microscope connected to a projection system.

To create a wet-mount slide of their cells, students will need to gently collect cheek cells.

1. Have each student take a swab or flat-sided toothpick and gently (no blood, please!) scrape the inside of their cheek.
2. Place a small drop of water in the center of a clean slide and smear the wet end of the swab/toothpick in the center of the drop.
3. Add one drop of methylene blue solution to the center of the slide.
4. Grasping it by its edges, GENTLY lower a coverslip over the sample, starting with one edge. Carefully soak up any excess moisture around the coverslip with a paper towel.
5. Place the slide on a microscope and observe using 4X or 10X objectives.

Stained cheek cells should look like light blue pancakes with a single dark blue dot in them. This dark blue dot is the nucleus. The nuclei turn blue because the dye sticks strongly to the DNA within them. This DNA contains copies of all the instructions to make another person (it just doesn't use them all in its job as a cheek cell.) Encourage students to record their findings:

- What do the cells look like? Can you identify the cell membrane? What else is obvious?
- Why do you think the cells are shaped the way they are?
- Could these cells live on their own? Why or why not?

Day 2

Part 2: Isolating DNA

The DNA in cells is like a recipe book that includes the instructions for making all the proteins and, therefore, all the structures and other molecules a cell needs throughout its life. These instructions are called genes and every gene codes for a protein. Of course, not every molecule in a cell is a protein (the genes, for example, are made of nucleotides) but proteins are important because they play so many roles in the cell. They can be structural, like the histones that hold together DNA in chromosomes. They can make chemical reactions go faster or slower. They even make all of the other molecules a cell needs to exist, like fats, carbohydrates, nucleotides, and even OTHER proteins. Given the amount of instructions it takes to keep even a simple cell going it's no wonder that DNA molecules are so large!

To isolate DNA from cheek cells students will be doing an alcohol precipitation. This is a technique used by labs all over the world to isolate DNA for further use, such as PCR and cloning. All alcohol precipitation protocols have three basic steps:

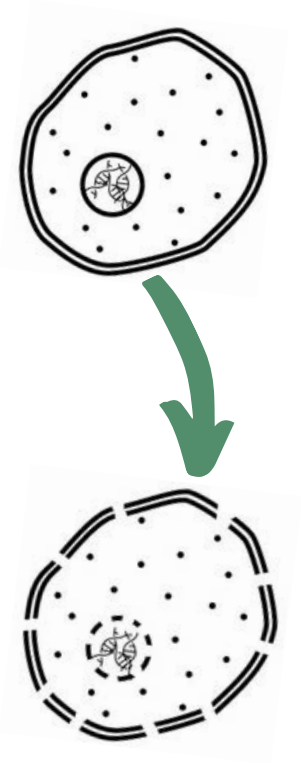
- Sample preparation: Students will collect their cheek cells by swishing a small amount of saltwater in their mouth for 2 minutes. This will give them a sample of cells in a buffered solution.
- Cell lysis: In this procedure, students will be using dish soap to rupture the cells by disrupting the membranes. They will also treat the samples with a protease (in this case: meat tenderizer) that will break down the peptide bonds in the cell's proteins and release the DNA.
- DNA precipitation: Ice cold alcohol (either isopropanol or ethanol) will cause the DNA to come out of solution and clump together.

The Protocol

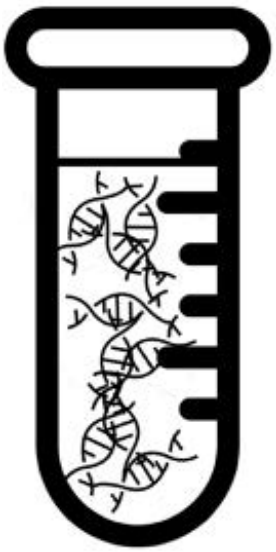
Instructor notes are in italics.

1. Sample preparation
    a. Take a small drink (5-10 ml) of saltwater and swirl it around for 1 minute. DO NOT swallow the water. Spit the water into the small cup.
        - *Make sure students are working only with their own cells. While nothing in this protocol is dangerous, remind students to be mindful of good health hygiene practices—you never know who has a cold!*
    b. Pour 5ml of the salt and cell solution into a 15ml tube. This is now a buffered solution that should contain lots of cells.
        - *This is best done before eating: you want DNA from the students, not their lunch!*
2. Cell lysis
    a. Add 20 drops or 1ml of dish detergent solution to the cell mixture. This will rupture the membranes in the cells. The detergent will disrupt the hydrophobic fatty tails of the phospholipids in the cell membrane.
        - *This should break down both the cell membrane and the nuclear membrane.*
    b. Cap the tube and mix the contents by gently inverting it for at least 30 seconds. DO NOT shake the solution! Bubbles and foam are bad.
        - *Encourage the students to gently invert this to agitate and mix the cells. Vigorous shaking will result in foam that disrupts later steps.*

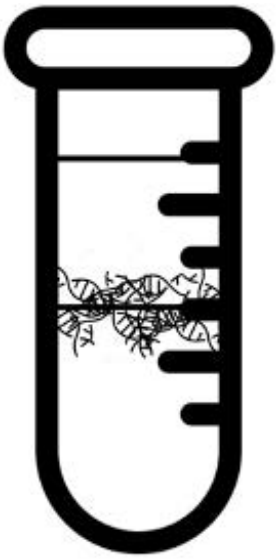
c. Remove the cap and add 20 drops or 1ml of the protease (meat tenderizer) solution. This will break down any residual proteins and release the DNA.
    - *Most meat tenderizers are a mixture of papain (from papaya fruit) and other salts. Unseasoned tenderizer works best for this. This is a great time to discuss why an enzyme that breaks down protein would make meat more tender. You can also use fresh pineapple juice for this or contact lens cleaning solutions made to remove protein deposits.*
d. Re-cap the tube and gently invert the tube for at least 30 seconds. Again: do not shake!



3. DNA Precipitation
    a. Hold the tube at a slight angle and CAREFULLY add 5ml of ice-cold alcohol by gently pouring it down the side. The alcohol should form a layer that floats on top of the cell buffer solution. A steady hand is the key to success!
        - *You may want to demonstrate this for the students before they try it. Ethanol and isopropanol will both work but they must be very cold. Keep the precipitation medium in the freezer until needed and then dispense into insulated disposable coffee cups.*
    b. Set the tube in a rack and leave undisturbed for one minute. Observe what happens.
        - *Students should begin to see a cloudy layer of "snot" form at the interface between the alcohol layer and the salt/cell layer.*



4. DNA Collection
    a. Add an additional 1ml of cold alcohol to a fresh tube. This is the storage tube for their isolated DNA.
        - *1.5ml Eppendorf tubes or similar work well for this.*
    b. Insert a clean bamboo skewer into the 15ml tube and gently turn it around in one direction. the DNA should spool around the skewer.
    c. Carefully remove the skewer with the DNA and immerse it in the smaller tube of alcohol. Dislodge the DNA by gently scraping it against the side of the tube. Students will be able to see their DNA floating in the alcohol as fine filaments.



Discuss and Report

Ask students to reflect as a group on what they saw when they observed the cheek cells and when they isolated their DNA.

- What parts were we able to identify in the stained cells? Why could we see them?
  The easiest part to see was the dark nucleus. The methylene blue stain preferentially sticks to the DNA in the nucleus, making it dark.
- Why did we swish with saltwater to collect our cells?
  The swishing loosened up the cells in our mouth and made them easier to remove. The salt water acts as a buffer to keep the cells intact until we're ready to break them down and release the DNA.
- What do the detergents and meat tenderizer do?
  The detergent breaks down the cell membrane and the membrane around the nucleus. The meat tenderizer is a protease—an enzyme that breaks down proteins. This strips protein off the chromosomes that would inhibit our ability to precipitate the DNA.
- DNA is very small and yet we can actually see it here. Why?
  DNA is a VERY long molecule. With enough DNA from enough cells, you can spool up the molecule into a visible chunk.

Career Exploration and Extension

Prompt students to think about and research what a career as a biomedical manufacturer or researcher might entail.

- What does a biomedical manufacturing operator do all day? What does Dathan do?
- What kind of training would a student need to manufacture biomedical products? What kind of training would they need to become the scientist who designs these systems?
- Are jobs like Dathan's in high demand? Will more people be hired to develop and manufacture new medicines in the future?
- What kind of education is needed to be a biomedical manufacturing operator? Where could a student be trained locally for this career? What types of classes are important?

Name: _____

Cheek Cells & DNA

Student Data Sheet

Dathan's work focuses on using eukaryotic cells in culture. This is similar to when scientists grow large cultures of bacterial cells, except these cells are more like the cells humans have in our own bodies—they have membranes around their nuclei and go about their cellular business differently than bacteria. The cells Dathan uses are not human cheek cells, obviously, but he does use cells derived from other animal tissues to produce proteins and other medical products.

Part 1: Examining Your Cheek Cells

In the first part of today's protocol, you'll be preparing a slide of your own stained cheek cells. First, it might be helpful to think about what you know about cells and DNA.

What are cells?

What is DNA? What does it do?

Where do cells keep their DNA?

Protocol: Slide Prep and Staining

1. Prepare your slide! Place a small drop of clean water in the center of a clean microscope slide and make sure you have a coverslip. This is where you'll put your cells.
2. Collect your cheek cells. Use a cotton swab or flat-sided toothpick to gently (no blood, please!) scrape the inside of your cheek. You should be able to see the cells as a whitish material.
3. Smear the wet end of the swab/toothpick in the center of your slide's water drop. You should see the white material spread out in the water droplet. This is good! You'll be able to see the cells better if they aren't all piled up together.

Name: _____

Protocol: Slide Prep and Staining, Continued

4. Obtain a dropper of methylene blue solution from your instructor and add one drop to the wet area in the center of the slide. The blue dye will stain the DNA in the cells, making it easier to see. Caution: This part may be messy and methylene blue will stain!
5. GENTLY lower a coverslip over the sample, starting with one edge. Carefully soak up any excess moisture around the coverslip with the edge of a paper towel.
6. Place the slide on a microscope and observe using 4X or 10X objectives. You will need to use the lower power lenses to find the cells before shifting to higher lenses.

Stained cheek cells should look like blue pancakes with dark blue spots in the center.

What do your cells look like? Can you identify the cell membranes?

Draw a picture of your cells:

Name: _____

Why do you think the cells are shaped the way they are?

Can you see the DNA (or at least where it's stored?) What lets you see the different parts of the cell?

Part 2: Isolating your DNA

In this section, you will be using a very common lab technique called an alcohol precipitation to isolate your own DNA from your cheek cells. The work Dathan does requires him to understand how DNA works and to be able to move DNA instructions from one organism to another. For this project, you'll be collecting DNA from your own cells.

Alcohol precipitation works because DNA is a charged, polar molecule. Alcohol is a non-polar solvent. Think about what happens if you drop some water into a glass of cooking oil: it will stay separated as a small bubble and won't mix. Water is a polar liquid, while oil is not so they do not mix or dissolve into each other. DNA acts the same way in alcohol and will wad up into a small, viscous ball.

Name: _____

Protocol: Alcohol Precipitation of DNA

1. Select a partner and gather all of your materials. Each person will need a larger 15ml tube for their precipitation reaction and a smaller 1.5 ml tube for storing their DNA once it is collected. Your teacher will provide the other reagents.
2. **Sample Preparation**
    a. Take a small drink (5-10 ml) of the provided saltwater and swirl it around your mouth for 1 minute. DO NOT swallow the solution. Spit the water into a cup. Note: DO NOT share cups with your partner! Everybody should do their best to keep their germs to themselves!
    b. Pour 5ml of the salt and cell solution into a 15ml tube. Your cheek cells should be suspended in this solution.
3. **Cell Lysis**
    a. Add 20 drops (or 1ml) of dish detergent solution to the cell mixture. This will break open the cell membranes.
    b. Cap the tube and mix the contents by **gently** inverting it for at least 30 seconds. DO NOT shake the solution!
    c. Remove the cap and add 20 drops or 1ml of the protease (meat tenderizer) solution. This will break down any residual proteins and release the DNA.
    d. Re-cap the tube and **gently** invert the tube for at least 30 seconds.
4. **DNA Precipitation**
    a. Hold the tube at a slight angle and CAREFULLY add 5ml of ice-cold alcohol by gently pouring or pipetting it down the side. The alcohol should form a layer that floats on top of the cell solution.
    b. Set the tube in a rack and leave undisturbed for one minute.
5. **DNA Collection**
    a. Add 1ml of cold alcohol to the small 1.5 ml tube. This is the storage tube for your isolated DNA.
    b. Insert a clean bamboo skewer (or straw) into the 15ml tube and gently turn it around in one direction. The DNA should spool around the skewer.
    c. Carefully remove the skewer with the DNA and immerse it in the smaller tube of alcohol. Dislodge the DNA by gently scraping it against the side of the tube.

You should be able to see your DNA floating in the alcohol as fine filaments!

Name: _____

Reflection

What did you observe after your DNA precipitation? Describe what you saw.

What do the detergents and meat tenderizer do the cells?

DNA is very small and yet you can actually see it in your small test tube. Why is this?

ACKNOWLEDGEMENTS

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Images

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