Standard Operating Procedure (SOP): Extracting Your Own DNA

Prepared by: Maarten Rotman, Ph.D. (Office of Entrepreneurship, Mayo Clinic, Jacksonville, Florida) (05/2019), contact: rotman.maarten@mayo.edu

Description of event:	Students isolate squamous epithelial cells from the outer epithelial layer of the mouth / inner cheek. They lyse these cells and precipitate the DNA, visualizing it as white strands, which can be transferred to a vial with (rubbing) alcohol to preserve and observe.
Purpose:	To demonstrate the concept of DNA extraction from human biological samples, exposing them to the scientific fields of biology, biochemistry, and genetics.
Length of the event:	20 – 45 minutes plus setup/cleanup. (the needed time can be flexible depending on the amount of independence of the students: the organizers can prep and pipet everything, the students can do it themselves, or anything in between, depending on the amount of time you have. For a group of 5 students, 20 minutes is a slightly rushed minimum)
Participants:	K12 (pref. 9-15 years old, adapt depth of explanation to age)
Items needed:	Table salt, potable water, SDS, 95% EtOH, isopropanol, pipets to transfer at least 5 and 10 mL of liquids, cups (e.g. 50 mL tubes), small vials (e.g. 5 mL round bottom tubes), small plastic spatulas, bucket with wet ice, tube rack, timer, gloves, lab-coat, tissues, markers (ideally alcohol resistant).

Scientific background

In this activity, students will extract their own DNA from the cells of their inner cheeks *(squamous epithelial cells from the outer epithelial layer of the mouth / inner cheek)*. DNA (deoxyribonucleic acid) is the backbone of the central dogma in biology, the blueprint of life on earth.

The students extract their cells by swirling around a salt solution in their mouths. Once back in a tube, the cells in this solution will be lysed by an SDS solution, after which ice-cold ethanol will be used to precipitate the DNA. The students will carefully remove the precipitated DNA and transfer it to a new vial that contains rubbing alcohol (isopropanol).

Detailed description of the event with practical notes

Set-up (1-2 h, depending on group size, the day before the activity).

1. Prepare the liquid reagents according to the table below. Keep the 95% EtOH on ice at least 2 hours before the experiment.
2. *Optional:* In case of limited amount of time for the experiment, big groups to be supervised at a time, or very young participants, prepare for each student a 50 mL tube with 10 mL 0.9% NaCl solution.
3. *Note:* be absolutely careful to keep the salt solution physically separate from the other liquids, and clearly label each container. This is crucially important as the students will take the salt solution into their mouths, and obviously you do not want to be that one teacher that gives their students 190% proof party shots.

Introduction (5 min): The organizers should explain what DNA is, where it is to be found, and why one would want to isolate it. Make it interactive by having the students come up with good reasons, such as medical diagnosis, genetic modifications, forensic science, paternal testing, etc. Some trivia to potentially include depending on the time constraints: red blood cells do not

have DNA, some cancer DNA can be detected free flowing in the blood and is the source of many new blood tests, one human nucleus contains about 6 feet (nearly 2 m) of DNA packed tightly around histones, and all the DNA in one adult human would be long enough to reach from earth to the sun and back 6 times.
The organizers should also briefly explain the procedure, and pay careful attention to explaining that the solution will be salty like sea water, and that once the SDS has been added great care needs to be taken not to be rough, as to prevent to destroy the DNA (otherwise it will be impossible to transfer the precipitate to the final container – this is the most common failure, but easily avoided). If the students are to pipet the solutions themselves, explain the way the pipets work. *Do not allow the students to pipet by mouth; we're in the 21st century.*

Activity: extract cells, precipitate DNA (* = can be prepared or done by organizers)

1. Divide students in groups of max. 5 per organizer.
2. Have the students wear gloves and a lab-coat.
3. Give the introduction, and make them label a 50 mL tube with their names or initials.
   See suggested introduction above.
4. *Have the students pipet 10 mL of 0.9% NaCl solution in a 50 mL tube.
5. Have the students take the 10 mL of 0.9% NaCl solution in their mouths, make them swirl it around for about 30 s, like mouth-wash.
   Remind the students that the more they swirl it around, the more cells they will get, and the easier it will be to obtain DNA later on.
6. Spit it back into the same 50 mL tube.
   This solution contains the cells.
7. * Pipet 5 mL 0.01% SDS solution on top of the 10 mL salt solution. Cap the tube.
8. Make the students carefully mix the salt water / SDS solution by slowly rotating the tube about 6 – 10 times.
   It is crucial not to be rough with the mixture from this point on. The cells will lyse, but the DNA should not shear under stress.
9. Place the tube into the rack, uncap the tube, and wait about 1 – 3 minutes.
   Now the cells are being lysed by the SDS. Do not leave this step for longer than 5 min. maximum.
10. * Pipet 5 mL ice-cold 95% EtOH carefully along the wall into the tube, in order to place a layer of EtOH floating on top of the layer of the salt water / SDS mixture.
    It is important not to mix the layers, do not pipet the EtOH straight into the liquid, and do not mix the tube.
11. Leave the tube standing upright in the rack for about 1 – 3 minutes.
    The DNA will now be precipitating into the EtOH layer, and should become visible as little white strands floating in the top layer.
12. * While it stands to precipitate, prepare a small vial by filling it with about 1.5 – 2 mL of isopropanol.
13. Using the plastic spatula, carefully swirl around in the EtOH only, to collect the DNA.
    It is very important not to agitate the underlying layer of salt water / SDS, nor to be rough while swirling. The DNA can disintegrate easily, making it nearly impossible to transfer it.
14. Transfer the collected DNA to the prepared vial with isopropanol.
15. Have the students label their vial with their initials.
16. Dispose of the waste appropriately, and collect the lab–coats.

Step-by-step preparation for the event

Step	Preparation and check-list	Time needed
1.	Obtain items needed for the experiment: - **Reagents listed in table below** - **Appropriately sized containers for the liquid reagents** (obtain from lab, or Amazon: https://www.amazon.com/dp/B01JM395T2) - **Paper towels** - **Table** - **Timer** - **Laboratory coats** (one/student; obtain from lab or a disposable one from Amazon: https://www.amazon.com/ dp/B07M7FFPGR) - **Gloves** (one/student; obtain from lab or Amazon: https://www.amazon.com/dp/B007N8WQDG) - **Tube rack** - **50 mL tubes** (one/student; obtain from lab, or Amazon: https://www.amazon.com/dp/B01M04HGPJ) - **5 mL plastic sample tubes** (one/student; obtain from lab, or Amazon: https://www.amazon.com/dp/B074V7T8HJ) - **Serological pipettes** (min. 5 mL, 10 or 25 mL advised; obtain from lab, or Amazon: https://www.amazon.com/dp/B07LCXHBLK) - **Ice bucket** (to be filled with wet ice 2 h before experiment) - **Alcohol resistant permanent markers** (obtain from lab, or Amazon: https://www.amazon.com/dp/B00JEEV1NI)	At least 1 month prior to the event (depends on how long it takes for products to ship; especially obtaining the alcohols may require more time than expected if this is not a normal order item for your location.)
2.	Try the experiment yourself. It is recommended that the organizers try the experiment at least once to get a better understanding of the process and to address any potential problems that may occur.	2-3 weeks prior to the activity
3.	Prepare the reagents, and set up the materials for students to use.	1 week - 1 day prior to the activity
4.	Place the 95% EtOH solution on ice.	2 h before activity.

Step-by-step activity protocol

Step	Preparation and check-list	Time needed
1.	Ensure all materials are present and 95% EtOH is on ice.	2 h before activity
2.	Students don lab coat and gloves	5 min.
3.	Give introduction while students label 50 mL tube	5 min.
4.	Add 10 mL 0.9% NaCl solution in each 50 mL tube	2 min.
5.	Have student swirl the salt water solution around in mouth	30 sec.
6.	Spit back in tube, add 5 mL 0.05% SDS solution	2 min.
7.	Mix solution carefully 6 – 10 times, then leave tube in rack for 1-3 min.	5 min.
8.	Lay 5 mL ice-cold 95% EtOH on top of mixed solution, rack 1-3 min.	5 min.
9.	During precipitation step, put 2 mL isopropanol in 5 mL sample tube	1 min.
10.	Pick op white strands of DNA, move to sample tube.	5 min.
11.	Label sample tube, dispose of waste appropriately	1 min.

Reagents preparation

	Up to 45 students	Product number (FischerSci *)	*Per student*
0.9% NaCl in water			
Table salt (non-iodized)	4.5 g	N/A	0.09 g
Potable **water**	500 mL	N/A (use normal water)	10 mL
95% EtOH (190% proof)**		04-355-232	
EtOH, absolute	237.5 mL	BP2818500	4.75 mL
Deionized water	12.5 mL	N/A (normal water is ok too)	0.25 mL
0.05% SDS			
20% SDS	250 mL	BP1311-200	5 mL
Deionized water	0.63 mL	N/A (normal water is ok too)	0.09 g

** There is no need to stick with one particular vendor, this is purely an example.*

*** Note that if it needs to be bought just for this experiment, it is significantly cheaper to buy 190% proof EtOH compared to EtOH absolute.*