ISOLATING YOUR OWN GENOMIC DNA! LESSON PLAN.

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Time: ~1½ hours
Grade Level: Grade 6 and 7+.
Staff: 1 biologist/geneticist

Equipment: Saline solution, paper cups, SDS detergent, 95% ethanol, gel electrophoresis equipment, loading dye, etc (see below).

Genomic DNA is actually pretty easy to isolate using simple chemicals and household items. Here we present a streamlined protocol (and tips for a corresponding DIY alternative) for easy visualization of the students' own DNA. As well, in our SCLS[1] fieldtrip, we also provide an opportunity for students to load a DAN sample onto a gel electrophoresis set-up.

1. Set-up and supplies.

Below is the list of reagents and equipment necessary for the genomic DNA isolation experiment. Note that this lesson plan will also contain descriptions of possible DIY alternatives (i.e. if you do not have access to research grade materials).

Per student:
1 5ml saline pod (0.8% NaCl w/v)
1 dixie cup (small, paper)
1 disposable 15mL test tube
1 mL aliquot of 10% SDS detergent
10 mL of 95% ethanol
1 disposable plastic Pasteur pipets
1 microcentrifuge tubes

2 sets per class (one gel per ~15 students):
1.0% agarose gel
1X TBE Buffer
'DNA samples mixed with loading dye
2 sets of micropipettors (Pipetman)
2 sets of pipet tips

[1] See http://www.bioteach.ubc.ca/portfolio/science-creative-literacy-symposia/ (accessed April 22nd, 2015)

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2. Before the field trip starts (15 minutes)

Make sure all lab materials are in place. For example, put saline pods in the Dixie cups and rack the test tubes so that it will be easy for the students to grab their materials for the first step (the cheek cell rinse).

Prepare two agarose gels and set aside. These will be used after the genomic isolation.

3. Introductions (15 minutes)

Introduce yourself and your team, and then begin the session with a short introductory DNA primer lesson. Note that there is an accompanying slide show available for download at http://sciencecreative.wordpress.com. However, the key points that need to be addressed include the following:

1. DNA is found inside of the cells of all living things from microscopic bacteria to humans (including your favourite celebrities!) Most importantly, you (the students) have it too, and the purpose of this experiment is to isolate some of your own DNA.

2. DNA is the blueprint or instruction manual for cells. Essentially, this molecule represents a code, much like a form of text or language that ultimate results in the development of the tissue and/or organism. The entirety of the code necessary to provide these instructions is what scientists will often call the "genome."

3. This means that to make you, or any human for that matter, you'll probably need a lot of code (i.e. imagine describing every physical facet of a person – inside and out – by scribbling these notes on paper. Think how many pages of text would you need?). Anyway, it turns out that, on average, the amount of code (or letters of information if you will) to make a human is roughly 3 billion letters of code! This is about the same as 7000 copies of the first Harry Potter book.

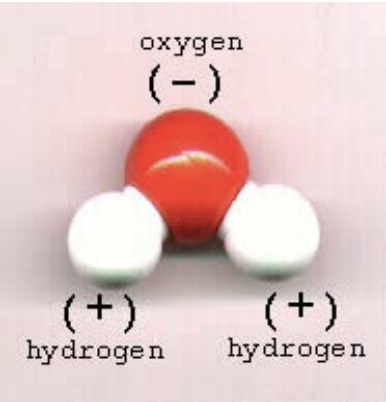
*4. (This section is optional, but depending on your comfort level and the enthusiasm of the students, you can try to incorporate more chemistry into the discussion). This lab is particularly good at introducing your students to some **biochemical** concepts since every reagent plays a particular role in either getting the DNA out of the cell, or to promote the **precipitation** of DNA. As a reminder, precipitation is the act of making things stick together. What then happens is that so many DNA molecules come together that they become this complicated aggregate - this aggregate can no longer stay soluble in the water and consequently will fall out of solution.*

*Firstly, however, there are a couple of traits about DNA worth mentioning. To begin with, since DNA is only composed of 4 different **nucleotides** (of which they are all very chemically similar to each other), you find that all pieces of DNA, whether they are short*

or long, will have the same general chemical characteristics. This has help tremendously in research because it just makes it quite easy to predict how DNA will behave in different circumstances.

For instance, since DNA has many ***phosphate groups*** *in its structure (lots of oxygens flailing around), you find that DNA is predominantly negatively charged (that's useful to know). Also, DNA is chock full of ring structures which are generally* ***hydrophobi****c in nature (water hating). This basically means that in certain circumstances, it likes to hang out with other things that hate water (kind of like when you see oil clump together in water) – this also means that it's not that easy to dissolve it in water (also very useful to know).*

Fortunately, aqueous (or water based) solutions are still pretty much the best solvents around since water has this AWESOME bipolar structure... (seriously, it's a thing of beauty, and also why folks get very excited when they find it on other planets).



Anyway, because DNA is usually swimming in this awesome liquid (say inside the cell), it is usually in "solution" (or dissolved) when you encounter it in nature.

5. Lecture should also end on a section highlighting safety as needed for the session (i.e. if you're in a lab). FYI: the only material that is involved in this fieldtrip with a minor hazard rating is the ethanol. You can share with the students that if they spill on their skin, it's o.k. although it will feel cold for a bit.

At this point, we can begin the experiment.

4. Protocol: Part I (10 minutes)

1) Break the top of the saline pod and squirt the saline (5ml) into mouth. Swish saline in mouth firmly but not too vigorously for 30 seconds. *If rinsing leads to the frothing of the saline, you're probably doing it too aggressively. This is actually o.k. for the DNA, but the bubbles will make things a little tougher to see in later steps.*

2) Spit "spit" into the Dixie cup.

3) Pour the cheek cell rinse from the cup into the test tube. Snap the cap of the test tube shut. Throw away the Dixie cup and empty saline pod.

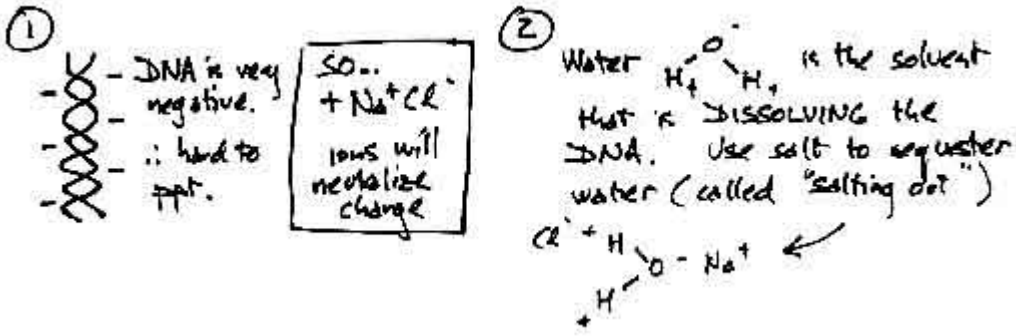
Note that depending on the space you are using, there may be strict rules related to these first couple of steps. Since they involve placing material in a student's mouth, you should check with your safety admin on permissions involved. For instance, with the Science Creative Literacy Symposia field trip (which makes use of an authentic research space, we are not allowed to do these first few steps in the lab, but rather perform them in the adjacent lecture hall).

Helpful Hint!
At the end of each step during this procedure, have each student hold up their tube and show it to you. This ensures that no one gets left behind. Tell them not to proceed with the step until you have explained it fully.

5. Protocol: Part II (30 minutes)

5) Remove the cap of the test tube containing the cheek rinse and pour the SDS aliquot into it. Snap or screw the cap securely shut. Invert tube *gently* 4-5 times. Allow cell lysis to proceed for at least 5 minutes (no more than 10minutes), by leaving it on the bench. If you like, you can use this time to explain all the biochemistry that is happening so far.

*Saline is all about the salt. We have plain old **sodium chloride** and its purpose is two fold. First, it's being used at a concentration that is very similar to what cells actually have (0.8% w/v). This means that when your cheek cells leave your cheek and get exposed to the saline, it's going to feel a bit like home to them. Secondly, the salt is also there to aid in the precipitation step. Here, it works by neutralizing the negative charge on the DNA molecules (the Na+ ions in particular), and also by interacting with the water thereby preventing the water from solvating the DNA molecule. Sort of like this...*



*But before any of this neutralization can occur, you actually have to get the DNA out of your cheek cells first! This is because you haven't done anything to **lyse the lipid membranes** of the cells. Your DNA is still inside!*

*This is what the **detergent solution** is doing. SDS or sodium dodecyl sulfide is a soap! A detergent is an emulsifier, which means it kind of looks like a lipid itself **(lipid is a fancy word for fat).** As you may remember, lipids have **hydrophilic** (water loving) and **hydrophobic** (water hating) regions. The presence of an emulsifier allows the breakage of these lipid membrane structures, which ultimately means that the DNA can get out of the cell.*

GAME INTERLUDE!

While waiting for cells to lyse, you can also try and squeeze in a little game. We often try to give students a somewhat oversimplified version of the "Central Dogma" of molecular biology to explain how DNA "instructions" are used in the body by "translating" DNA to protein. It also explains how changes in DNA (mutations) can lead to a physical change in phenotype. You can check this activity out in the corresponding slide at http://sciencecreative.wordpress.com

6) Remove the cap and slowly pour in the ethanol. Pour along the sides trying not to mix the ethanol with the rinse + SDS. You want a layer of ethanol on top of the rinse + SDS mixture. A stringy precipitate should form at the interface between the two liquid layers.

***Overlaying with Ethanol:** Here's the set-up. DNA is currently swimming around in a **sea of water with the salt neutralizing its charge and sucking up water**. DNA is also hydrophobic, so really it's just itching to clump together and precipitate out. In fact, if you were dealing with a high concentration of DNA, it may have already done so (you might already see some white stuff).*

*However, if it hasn't precipitated, then adding ethanol into the picture will usually do the trick. This is because it changes the environment that the DNA interacts with. Since **ethanol** (in comparison with water) is a **crappy solvent,** when DNA comes into contact with the alcohol, it will not want to stay solvated and instead will begin precipitating. At which point, you will see something that looks like white stringy snot, mostly at the interface of the two fluids.*

7) Use the disposable pipet to transfer the DNA precipitate to a microcentrifuge tube. Make a big deal of the technique. **Students have problems at this step!** They often end up shearing their DNA and missing the bulk of it because they squeeze the bulb repeatedly and at the wrong time.

8) Have them label their tubes and put them somewhere so that they can be taken home.

DIY version:

A fairly good genomic isolation experiment can also be performed by starting with a different tissue source, and using grocery store reagents. How much of each solution you

use will mostly depend on the dimensions of the drinking glass or beaker. In other words, you want to have comparable amounts to easily visualize the alcohol layer sitting on top of the salt water layer. The main substitutes include the following:

- 8g table salt prepared in 100ml of bottled water (instead of the saline pod).

- Use of a small drinking glass (instead of a 15ml tube). Hence why the amounts mentioned above have been scaled accordingly.

- Use a dishwashing detergent (instead of SDS), which you will add at approximately 5% v/v (i.e. 5ml per 100ml of salt solution). Note that the more simple the detergent, the better (i.e. avoid scented or coloured versions if possible).

- A portion of strawberry, an equivalent sized chunk of banana or amount of wheatgerm (instead of the cheek cells). Note that because we are using plant cells, it's usually a good idea to include a physical step (i.e. squishing with a chopstick), whilst the plant material, salt water and detergent are mixed together.

- Use high percentage rubbing alcohol – this is usually available at most pharmacies at 98% (instead of the 95% ethanol).

6. Gel Electrophoresis (<30 minutes)

This part of the lesson will require some specialized equipment: namely, a gel electrophoresis unit and knowledge of how to use such equipment. There is actually a DIY version, but it is quite finicky – you can find out more at http://www.scq.ubc.ca/the-macgyver-project-genomic-dna-extraction-and-gel-electrophoresis-experiments-using-everyday-materials/

Split class into two groups for this part of the workshop. Explain to them that we have some "DNA samples" to load. These can be from a previous class, or they could simply be placebos. They will be prepped with loading dye (glycerol plus bromophenol blue dye) to facilitate loading.

Before starting, highlight a number of key concepts. These might include:

1. How the principle of electrophoresis generally works, and specifically in terms of how it separates DNA molecules according to size.

2. Pipetman technique and "etiquette."

PROTOCOL:
1) Pour TBE buffer into pre-cast gel.

2) Demonstrate how to load the gel.

3) Have the students line up single file and take turns loading the gel.

4) Put on the lid to the gel chamber and turn on the electrical current. Turn the voltage down to 60 or so. The gel should run throughout the remainder of the day. You typically will not have time to look at it at the end of the day, so occasionally, we will actually just use placebo samples (the students love loading the gel anyway).

7. And that's a wrap! (<5 minutes)

Thank the class for their hard work, and as a wrap up (if there's left over time), you can ask the class if they have any DNA related questions.