Double DNA Extraction Lab; (SB2 a,b,f)

Part 1: Human Cheek Cell DNA

Purpose: (read the below summary and then write your own purpose)

Introduction: DNA…you hear about it all the time. DNA is used every day by scientists and lawyers to help in criminal investigation, paternity suits, cloning, etc. Your DNA is your "genetic fingerprint"—this means that your DNA is like no one else's in the world! The procedure that we will use to see your DNA includes the same basic processes that researchers use to isolate, analyze, and manipulate DNA in a laboratory setting (although the DNA isolated here is not nearly as "pure" as the research lab version).

DNA is a nucleic acid made of carbon, hydrogen, oxygen, nitrogen, and phosphorous. DNA can be considered the hereditary "code of life" because it possesses the information that determines an organism's characteristic and is transmitted from one generation to the next. You receive half of your genes from your mother and half from your father. Day to day, DNA's job is to direct the functioning within the cells of your body.

DNA is in the nucleus of almost every cell in your body. The length of DNA per cell is about 100,000 times as long as the cell itself. However, DNA only takes up about 10% of the cell's volume. This is because DNA is specially packaged through a series of events to fit easily in the cell's nucleus. The structure of DNA, the double helix, is wrapped around proteins, folded back onto itself, and coiled into a compact chromosome.

Individual chromosomes can be studied using microscopes, but the double helix of a chromosome is so thin that it only be detected through innovative, high-tech procedures. Chromosomal DNA from a single cell is not visible to the naked eye. However, when chromosomal DNA is extracted from multiple cells, the amassed quantity can easily be seen and looks like strands of mucous-like, translucent cotton.

We will first collect cheek cells by swishing a sports drink in our mouths and using our teeth to scrape cells off our cheeks. (The more vigorous and the longer that you swish, the more cells are removed, and the more materials you'll have from which to extract DNA.) Then, we will lyse the cell membranes by adding a detergent based cell lysis solution, which allows the DNA to be freed. DNA is soluble in water, but much less soluble in alcohol. Thus, alcohol will be slowly added, and DNA will precipitate to the sports drink/alcohol interface, and you will be able to see your own DNA! The white, stringy material is thousands of DNA molecules stuck together (with some proteins too). We will then perform a similar extraction of eukaryotic DNA from a member of the Plant Kingdom, strawberries! Now, write a purpose for this lab using the above information.

Materials and Methods

1. Obtain a glass bottle and cap and thoroughly rinse three times.

2. Obtain a small cup (~10 ml) of sports drink and swish it around in your mouth for 1-2 full minutes. As you swish, gently and continuously scrape the sides of your cheeks with your teeth to help release your cheek cells.

3. Spit the drink (with your collected cheek cells) back into the small cup

4. Pour the contents of the cup into your glass bottle (discard the cup).

5. Holding the bottle at an angle, use the provided plastic pipet to add 5 mL of cell extraction lysis solution to your collected cheek cells.

6. Cap your bottle and gently invert it 5-8 times. (This mixes the lysis solution with the cheek cells.) DO NOT SHAKE!

7. Allow this to stand for 2 minutes.

8. Using the provided pipet, SLOWLY add 10 ml of the ice cold alcohol *by letting it run gently run down the side of the bottle (hold the bottle at an angle)*. You should have 2 distinct layers. DO NOT mix the cheek cell solution with the alcohol!

9. Watch as wispy strands of translucent DNA begin to clump together where the alcohol layer meets the cheek cell solution and float to the top. (It kind of looks like cobwebs extending upward.)

10. Place your bottle in a designated spot and let it stand undisturbed for 5 minutes. During this time the DNA will continue to precipitate out. During this time answer questions 2-3 of the discussion questions.

11. Next you will use glass rod to transfer your DNA into a micro-centrifuge tube. Melt the glass rod under an open flame to create a small hook. Next, spool the DNA out of the glass vial and place a small amount onto a slide. Make a wet mount of the DNA and view under a microscope. You might need to stain the DNA with iodine to help you visualize the DNA.

Discussion Questions (Answer some questions during part 1 and the rest during part 2)

1. Sketch the DNA under the three powers of magnification using the techniques practiced in the Introduction to Microscopy Lab. Draw and label the field of view for each sketch.
2. Sketch and label the three components of a nucleotide.
3. Describe the two major functions of DNA polymerase and relate these functions to show how cells help to ensure that DNA replication is accurate?
4. Describe how long strands of double-helical DNA fit into the nucleus of a single cheek cell.
5. Why can the mRNA strand made during transcription be thought of as a mirror image of the DNA strand from which it was made?
6. If a DNA segment has the nucleotides AGCCTAA, what would be the nucleotides of the complementary RNA strand?
7. Explain why DNA is referred to as your genetic fingerprint?
8. Explain why we used detergent to help us extract the DNA and justify your answers.
9. Thoroughly describe what is meant by “One gene, one protein”. You will need to outline the three major steps of protein synthesis in your answer.

OVER Part 2→

Part 2: Strawberry DNA Extraction

Now that you have extracted DNA from human cheek cells, you will use a similar technique to extract the DNA from another eukaryote, strawberries. You will learn in standard SB1c that human somatic cells are diploid, which means they have two sets of chromosomes, one from each parent (2n = 46 chromosomes). In fact, most eukaryotes are diploid; however, strawberries are actually octoploid and have eight sets of chromosomes (8n = 56 chromosomes). You should expect to get quite a bit of DNA from you extraction.

Materials and Methods:

1. Place one strawberry into a ziploc bag seal shut and squish for a few minutes to completely macerate the fruit and expose cells.

2. Add 8 ml of cell extraction lysis solution and squish for a few more minutes. Try not to make a lot of soap bubbles.

3. Filter through a moistened paper towel or coffee filter set in a funnel, and collect the liquid in a clear tube or bottle. *Do not* squeeze the paper towel. Collect about 3 ml liquid.

4. Add 6 ml of ice cold isopropyl alcohol to the strawberry liquid in the tube or bottle. Pour the isopropyl alcohol carefully down the side of the tube so that it forms a separate layer on top of the strawberry liquid.

5. Watch for about a minute. Once again, you should see a white fluffy cloud at the interface between the two liquids. Let the bottle stand undisturbed for 5 minutes to allow time for the DNA to fully precipitate out. During this time continue answering post lab questions for part one.
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6. Next you will use glass rod to transfer your DNA into a micro-centrifuge tube just like you did in part one. Make a wet mount of the DNA and view under a microscope. You might need to stain the DNA with iodine to help you visualize the DNA.

7. Rinse both the funnel and the bottle three times. Put the ziploc bag and paper towel in the garbage and return the room to homeostasis.

Post Lab Analysis Questions: (continued from part 1 questions)

10. Compare and contrast the appearance of the cheek DNA with the strawberry DNA when viewed under the microscope.

11. Read and write a brief summary on the article on the back side of this paper titled “World's largest genome belongs to slow-growing mountain flower”.

12. In the article the authors discuss DNA “base pairs”. What is meant by the term “base pairs”?

13. The article also using the term “genome”. Describe this term.

14. Do you believe that because the bacterium called *Carsonella ruddii* has the least number of base pairs of any living organism, it would also have the least amount of proteins? Please justify your response.

World's largest genome belongs to slow-growing mountain flower

An unremarkable and slow-growing plant has stunned scientists after they found it had the world's largest genome – 50 times bigger than that of our own species.

It is an unremarkable and rather fragile plant, but a flowering herb that frustrates gardeners for being notoriously difficult to grow has stunned scientists after they discovered it has the world's largest genome.

The DNA contained within Paris japonica dwarves all other plant and animal genomes that have been analysed so far. It is 50 times longer than the human genome, even though our species is thought to be one of the most complex and advanced on the planet.

A genome - the biological code which directs how every organism develops - is made up of the DNA found inside almost all living cells.

The slow-growing plant, which is native to the mountains of the Japanese island of Honshu but is also found in gardens in the UK, boasts more than 150 billion base pairs – the basic building block that links together to form DNA – in its genome. Humans have just three billion base pairs.

If stretched out, the genetic information contained within just one cell of Paris japonica would stretch more than 328 feet – taller than Big Ben – while the genome from a human cell would stretch just 6.5 feet. The discovery has added to questions about why our own genome is so comparatively small. It may also lead to the discovery of new types of drugs.

Dr Ilia Leitch, a geneticist at Kew Gardens' Jodrell Laboratory who was part of the team that made the discovery, which is published in the Botanical Journal of the Linnean Society, said: "When we started looking at the plant on our machine to measure genome size it was clear there was something odd about *Paris japonica*.

"When we worked out just how big it was, I was staggered. I had to keep checking to be sure. It is amazing how the cells pack all of that DNA in there.

"Other members of the family that Paris japonica is part of have medicinal qualities so there could well be some that we can identify from the genome.

"Genome size does not necessarily relate to the complexity of an organisms. We can now start probing what is going on in these really big genomes and understand what is going on.

"It can tell us what restricts where plants can grow and how they are going to respond to climate change. While many factors affect how and where plants grow, it now seems that genome size is one of the factors."

The smallest genome found so far belongs to a bacterium called *Carsonella ruddii*, which has fewer than 160,000 base pairs. The smallest plant genome belongs to a carnivorous plant called *Genlisea margaretae* with 63.4 million base pairs, which if stretched out would measure just 1.6 inches. The previous record holder for the largest genome was the marbled catfish, which had 130 billion base pairs and would stretch 288 feet. Dr Leitch said that having such a large genome could help explain why *Paris japonica* was so slow-growing and vulnerable to pollution and other extreme conditions. She added: "Having that much DNA does have consequences for the plant – plants with big genomes are at greater risk of extinction, more sensitive to pollution. Having so much DNA means every time you want to divide a cell in order to grow, you have to replicate all of that DNA, which takes time.