Grade: Grade11/12	**Subject:** Biology	**Unit:** Genetics/Molecular Biology
Title: Separation Exploration – The Science of the Separation of Mixtures		

Rationale

Mixtures frequently need to be separated in biology, biochemistry and chemistry. The scientist might need to separate one type of antibody from many, separate pieces of DNA from another by size & charge, or need to purify a protein for later use. All of these laboratory techniques come from the concept taught in Grade 9 Science about the separation of mixtures based on physical and/or chemical properties. Later, in Grade 11 and/or Grade 12 Biology, the concept comes up again in gel electrophoresis. These concepts are re-visited and expanded upon in university chemistry courses, such as analytical chemistry. These analytical techniques are widely used in industry.

Background Information

The Wakarchuk lab at Ryerson University (under the umbrella of GlycoNet) is a carbohydrate research lab that uses enzymes for therapeutic purposes. Some of these purposes are:

- The use of enzymes to add sugars to protein drugs to make these drugs last longer (persist) in the bloodstream, thereby reducing dosage, side effects and cost
- Adding sugar molecules to cultured neuronal cell surfaces using enzymes, so that these modified neurons will find their way to where they are needed when placed in Parkinson's patients
- Researching how to make these proteins using bacteria (rather than maintain expensive mammalian cell lines)

In this laboratory, there are many techniques that are used to separate mixtures and/ or analyze them. These techniques include:

- FPLC (fast protein liquid chromatography)/HPLC (high performance liquid chromatography)
- Gel electrophoresis and SDS-Page
- TLC (Thin Layer Chromatography)

For further information about the research done at the Wakarchuk lab, visit: https://www.wakarchuklab.com/

Curriculum Connections:

Grade 11 Biology: Genetic Processes

- D1.1 analyse, on the basis of research, some of the social and ethical implications of research in genetics and genomics (e.g., genetic screening, gene therapy, in vitro fertilization) [IP, PR, AI, C]
- D1.2 evaluate, on the basis of research, the importance of some recent contributions to knowledge, techniques, and technologies related to genetic processes (e.g., research into the cystic fibrosis gene; the use of safflowers to produce insulin for human use) [IP, PR, AI, C]
- D3.4 describe some genetic disorders caused by chromosomal abnormalities (e.g., non-disjunction of chromosomes during meiosis) or other genetic mutations in terms of chromosomes affected, physical effects, and treatments
- D3.5 describe some reproductive technologies (e.g., cloning, artificial insemination, in vitro fertilization, recombinant DNA), and explain how their use can increase the genetic diversity of a species (e.g., farm animals, crops)

Grade 12 U Biology – Biochemistry

- B1.1 analyse technological applications related to enzyme activity in the food and pharmaceutical industries (e.g., the production of dairy products; breadmaking; the use of enzymes to control reaction rates in pharmaceuticals) [AI, C]
- B2.1 use appropriate terminology related to biochemistry, including, but not limited to: active and passive transport, covalent and ionic bond, allosteric site, substrate, substrate-enzyme complex, and inhibition [C]

Grade 12 U Biology – Molecular Biology

- D2.3 conduct an investigation to extract DNA from a specimen of plant or animal protein [PR]
- D3.4 explain how mutagens, such as radiation and chemicals, can cause mutations by changing the genetic material in cells (e.g., the mechanisms and effects of point mutations and frameshift mutations)
- D3.5 describe some examples of genetic modification, and explain how it is applied in industry and agriculture (e.g., the processes involved in cloning, or in the sequencing of DNA bases; the processes involved in the manipulation of genetic material and protein synthesis; the development and mechanisms of the polymerization chain reaction)

Lesson Objectives/Concepts

- Students will learn about analytical techniques used in industry in a Canadian research laboratory located in Toronto, ON (Ryerson University) by listening to video clips from Canadian researchers discussing their current carbohydrate research
- Grade 11/12 U Biology students can choose to do a DNA Extraction Inquiry

Time

Period 1: 30 minutes in the computer lab & 30 min reading chosen article
Period 2: DNA Extraction inquiry: full period

Materials:

Period 1: Video clips & Literacy connection

- Students should bring their own earphones for use in the computer lab (or listen to the videos at home)
- Computer files:
  - Separation Exploration - Gr. 11/12
  - Ink Chromatography lab report & rubric

Period 2: Materials for **DNA Extraction Inquiry**

- Soft Fruit (mango, bananas, kiwis, dragonfruit, avocado, etc)
- Filters (cheesecloth, filter paper, mesh, stockings, filter paper, etc)
- Chilled rubbing alcohol – about 10 mL per group (4 degrees Celsius, fridge temp)
- 2 Beakers
- Elastic band
- Large test tube (25 ml)
- Mortar and pestle (1 per group) or the fruit can be mashed inside a Ziploc bag
- Salt water (1 tsp dissolved in 0.5 cup of water)
- Detergent (1 tsp per 1 banana)
- Wooden splint or wooden coffee stir stick

Overview

Period 1 (library period)
Part A: Listen to the Scientists

- Students use “Separation Exploration - Gr. 11 & 12” to guide them through the video

clips of the GlycoNet researchers.

Part B: Literacy Connection

- Read the June 9, 2017 CNN article "The Shifting Science of DNA in the Courtroom" by Channon Hodge: http://www.cnn.com/2017/06/09/health/dna-technology-forensic-evidence/index.html
- Students complete a chart that has them looking On the Lines (5 Ws and How), Between the Lines (inferencing) and Beyond the Lines (consequences).

Period 2: Grade 11 & 12 Biology: DNA Extraction Inquiry (full period)
Students begin the period by reading the experimental procedure from the *Scientific American* article and discussing the pre-lab questions either as a class or in small groups. Students will then carry out the DNA isolation experiment, and then finish the period with a final debrief to discuss the results, think of ways to vary the experiment, and make connections to current research in the glycomics field.

Activities/Procedure for Part 2:

Pre-teaching, done in advance of this lesson:

- Parts of a cell, components and structure of DNA

Grade 11 & 12 Biology: DNA Extraction Inquiry (full period)

Pre-lab questions:

1. What is the purpose of adding the detergent to the mashed up fruit?
   The purpose of the detergent is to break down the cell wall and nuclear membrane to release the DNA. In regular everyday life, we use detergent to break up the fats when washing dishes. It does the same thing here, breaking up the phospholipid bilayer of the cellular membrane.

2. What is the purpose of adding the salt to the mashed up fruit?
   It neutralizes the charge of the phosphate, making it more hydrophobic in water and helps it to separate better.

3. What is the purpose of adding cold alcohol?
   DNA precipitates at the fruit/alcohol interface.

4. Now that the DNA is extracted, what further testing can be done with it?
   Once DNA is released from the nucleus, one can cut up the DNA with enzymes and (theoretically) do gel electrophoresis to separate the pieces of DNA.

Directions to the teacher:

Read over the following article to get an idea of one procedure for the DNA extraction inquiry.
https://www.scientificamerican.com/article/find-the-dna-in-a-banana-bring-science-home/

1. Show the students how to do the general set up.

   Challenge the students to optimize this DNA extraction. You are in another country and you have to do a DNA extraction as part of a study abroad program. Some materials that you find easily in Canada (coffee filters, strawberries) at this time of year are not so easy to find. Instead of feeling defeated, you challenge yourself to complete the task with other materials that might be around that have the same function. Can you still do the extraction?

2. Have students brainstorm what they can use instead of coffee filters and strawberries. Encourage them to think about the important aspects. Coffee filters strain things so that smaller particles stay in the filter and the rest goes through. Some answers may include:

Alternatives to coffee filters

- Stockings
- Mesh cut from a laundry bag used for delicates
- Mesh used for covering some fruit
- Laboratory filters
- Cheesecloth

Alternatives to strawberries (soft fruit)

- Kiwis
- Dragon fruit
- Mango
- Canned fruit (pureed mango)
- Peaches
- Nectarines

Alternatives to dish detergent

- Shampoo
- 2 in 1 shampoo
- hand soap
- can change the amount of detergent
- can change the amount of salt

Summary

As a class, students view "The GlycoNet Story" video to get the context behind carbohydrate research and why it is so important for human health (therapeutic proteins, biofilms, etc.) and energy needs (biofuels). Individually, they listen to and answer questions about different researchers talk about the biochemical analysis that they do. Techniques such as gel electrophoresis, TLC (Thin Layer Chromatography) and FPLC (Fast Protein Liquid Chromatography) are discussed. After this introduction, students read about the context and impact of using DNA analysis in the courtroom. The following period, students continue with this theme by doing a DNA Extraction Inquiry.

Assessment: Computer file, "DNA Extraction Inquiry Biology 11 & 12 SR (student resource);" the narrative report outline and rubric from the ink chromatography lab (Grade 9) can be used for this activity as well.

Extensions :

1. DNA Day with Curio City – DNA and Food Security: http://curiocity.archive.letstalkscience.ca/Explore/ArticleId/5321/dna-day-2017.aspx

2. Use the same part A (video clips) and Part B (literacy piece) but do a water purification inquiry and connect to 11U Chemistry (Solutions and Solubility) with connections to the many issues surrounding water purification, treatment and distribution around the world.

3. Students can do use Gel Electrophoresis (Grade 12 Biology), using a virtual lab first and then a wet lab.

4. While studying photosynthesis, instead of doing the usual spinach chromatography lab, you can do the separation of the pigments in autumn leaves as a variation. Here is a lab activity intended for home use from *Scientific American*. https://www.scientificamerican.com/article/bring-science-home-leaf-colors/

5. Read about the "Biochemistry of Autumn Colours" on the famous Professor Shakashiri's Science is Fun webpage: http://scifun.org/chemweek/PDF/Fall_Colors.pdf or the high level content found here: https://staoblog.org/2014/10/13/world-science-festival-the-biochemistry-of-autumn-colors-world-science-festival/

References

- Nakita Beunbrazo, July 2017. Presentation for TDSB (Powerpoint presentation). Wakarachuk laboratory, Ryerson University.
- Dr. Warren Wakarchuk, July 2017. Presentation for TDSB (Powerpoint presentation). Wakarachuk laboratory, Ryerson University.

Narrative Laboratory Report[1]

Rationale: The idea behind a narrative laboratory report is to move students beyond a question and answer style of analysis or even a formal laboratory report. They have to ask themselves the core questions: What did I look for? How did I look for it? What did I find? What did it mean?

It is hoped that by asking students to step back and take a wider view of their work, they will move towards open inquiry where they begin to wonder and generate their own questions to answer (and being comfortable with that). After the initial uncertainty about marks and stepping out of their comfort zone, students generally like the freedom to work in this way, if the topic captivates them. It is the responsibility of the teacher to select opportunities for the students that have an interesting context or some aspect that is fascinating.

What was I looking for? (Describe your research question here along with your hypothesis) • Should be 1-2 sentences, includes a testable question & hypothesis
How did I look for it? (Describe your method) • Should be reproducible and step-by-step
What did I find? (Show any observations that you had) • Should include tables (succinct & organized with headings) and graphs (label axes, include title, legend if necessary)
What does this mean? (Analysis and Conclusion) • Should include analysis of scientific observations and data; should discuss results and **<u>why</u>** they occurred • Written in paragraph form

[1] Modified from Lenape District High School's Narrative Lab Report: https://www.lrhsd.org/cms/lib/NJ01000316/Centricity/Domain/26/templatepercent20narrativepercent20lab.pdf

Rubric for Narrative Report

	Level 1	**Level 2**	**Level 3**	**Level 4**
What was I looking for?	Research question did not include a properly testable question	Research question was somewhat focused with a testable question	Research question was well focused with a testable question	Research problem/question was highly focused with a testable question
How did I look for it?	Method was incomplete	Method was mostly there	Method was written in a generally reproducible way	Method was written in a detailed and highly reproducible way
What did I find?	Observations were present but were not organized/succinct; graphs lacking axes, labels & title	Observations were somewhat organized & succinct with table headings, good graphs	Observations were well organized & succinct with table headings, excellent graphs	Observations were highly organized & succinct with table headings, excellent graphs
What does this mean?	Analysis did not focus on the key question and did not address scientific error	Analysis somewhat captured the key questions but did not address the scientific error well	Analysis generally captured the key questions and addressed most of the scientific error	Analysis captured the key questions and worked in scientific error in a high level way

Answer Key

Part A: Listen to the Scientists

The GlycoNet Story video: https://www.youtube.com/watch?v=CQGEGIoqTpQ

Let the video play.Stop at the 1 minute mark.

1. What is the importance of understanding glycomics (the study of sugars)?
   Communication by cells or invasion into cells is often mediated by sugars. If we understand glycomics, we can interfere with infection processes.

2. What is GlycoNet?
   GlycoNet is a pan-Canadian network of researchers that formed in 2015 to develop solutions to unmet health needs.

3. Why bring these researchers together?
   This pooling of efforts should result in a streamlined process to get products to market as well as compete more effectively on the international stage.

Play video from 1 min – 2:47

4. What kind of good can result from these GlycoNet partnerships?
   Anything ranging from new vaccines to new medicines for diseases that are currently untreatable.

5. What areas have they made progress in?
   Influenza and diabetes

Meet the GlycoNet Scientists from Ryerson University, Wakarchuk Laboratory. Go to www.glyconetchromatography.weebly.com

Video clip#1: Tasnim 1 – Graduate Student

1. What is Tasnim's area of research?
   Her area of research is enzymatic synthesis.She attaches certain components onto proteins, carbohydrates or other biomolecules that the lab needs.

2. Why would Tasnim fluorescently label/tag molecules? Research this.
   It is not mentioned in the video but when you are trying to purify a molecule, it is beneficial to be able to see the desired product.In this case, she is visualizing the molecules with a fluorescent dye.

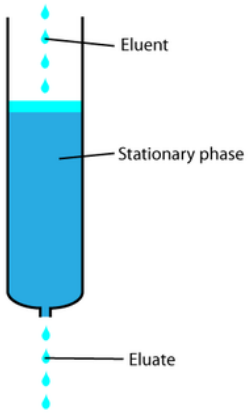
Video clip #2: Tasnim 2 – A typical day for Tasnim Abukar

1. Tasnim mentions that she uses TLC (Thin Layer Chromatography) on a typical day. What is TLC? What is it used for?
   TLC is used to separate mixtures into individual components. With paper chromatography, an ink can be separated into the particles that compose it. For example, if purple ink were dotted onto a piece of filter paper and dipped into water or another solvent such as alcohol or acetone, the solvent would move up the paper through capillary action and carry the ink particles with it. Since the ink particles differ in some way (size, charge, attraction to the paper, etc.), they move different amounts and can be seen separately. For more information, refer to http://chemguide.co.uk/analysis/chromatography/thinlayer.html.

2. What did Tasnim say was the basis of the separation in the TLC?
   She said that the molecules would separate based on polarity.

3. Tasnim mentioned that another technique that she uses a lot is FPLC (fast protein liquid chromatography).What is this?
   Chromatography involves the separation of mixtures into their individual components based on different properties of the components. It is used a lot in biotechnology and is similar to HPLC (high performance liquid chromatography) but at a high pressure. The separation could be due to size, charge or hydrophobic interaction.
   Reference: http://fplc.weebly.com/background.html

4. What is elution?
   In protein column chromatography, a solution containing the desired protein (plus impurities) is poured through the column. The protein will stick to the material inside of the column and the impurities will flow through. Later, another solution will be poured through to release the desired protein, which is then collected in pure form for further use. This process of "stripping" the column is called elution and is commonly used in biotechnology and biochemistry.
   Reference: https://en.wikipedia.org/wiki/Elution



Video clip #3: Ray Martinez-Rodriguez - Summer student (1:10 minutes)

1. What does the enzyme that Jose works with do?
   It moves sugars from a donor molecule to an acceptor molecule.

2. What are two problems with this protein (enzyme)?

One problem is that it is hard to purify and the other is that it is not very active.

3. Jose describes how a His tag can help fix one of the two problems mentioned above. What does it help with? Do an Internet search for His-tag (histidine tag) purification and IMAC (immobilized metal affinity column).He mentions using a nickel column. Draw a picture of an IMAC column and explain.
   Scientists add histidine to proteins to help to tag them for a purification process. When trying to scale up a substance for large scale production, it is important to be able to identify and purify the final product. This helps with the purification process. See question #4 for the rest of the answer.

4. Immobilized lactase in a calcium alginate gel and pouring milk through it to break down the lactase is a similar idea to the IMAC mentioned above. How are these two procedures similar and how are they different?
   The repeating His (histidine) tag has a certain affinity for metals such as nickel which are embedded in the column. A sample that is histidine-tagged can be poured through the Ni column, sticking to the column while impurities are flushed out. The his-tagged sample can be later recovered by eluting with another substance such as imidazole, which competes with histidine for the nickel. In this way, a recombinant protein can be recovered.

   This is different from using immobilized lactase since lactose passes through and the enzyme breaks it down. No eluting is necessary since the goal is to get rid of the offending lactose by breaking it down. The breakdown products are still present in the lactose-free milk. The two processes are different because the goals are different. IMAC uses affinity to remove a certain molecule from other molecules in the lysate. Immobilized lactase carries out a hydrolysis reaction on one component of the mixture and then releases the products back into solution.

   The two processes are similar because in both cases, a mixture of substances is poured through the column. With IMAC, one component is removed. With immobilized lactase, lactose is removed by breaking it down into other molecules.

   Reference: “How does His-tag purification work?” http://www.bio-rad.com/featured/en/his-tag-purification.html

Video clip #4: Dr. Ting Du - Post Doctoral Fellow (26 seconds)

1. Dr. Du talks about purifying her target protein. How does she do that?
   She does this with FPLC (fast protein liquid chromatograph) or AKTA (a protein purification system).

2. She also talks about constructing an operon. What is an operon?
   An operon is a cluster of genes that codes for a particular protein that is regulated by a single promoter.

3. What is the function of the proteins in the operon that she is talking about?
   These enzymes put an O-glycan (chain of carbs) onto her target protein.
   References:
   1. http://fplc.weebly.com/uses.html.
   2.https://www.britannica.com/science/operon

Video clip #5: Laura Kell, Research Technician (58 s)

As Laura talks about her job, she mentions two main responsibilities. One of them is training and mentoring other team members and assisting them to push the projects along.

1. Is it surprising to you that a research technician has this responsibility as part of their job?
   Answers will vary.

2. What is the other part of her job?
   Her research includes using lab techniques such as protein purification using FPLC (a form of liquid chromatography to analyze and purify proteins).She also uses enzyme assays with fluorescently labeled substrate (a molecule that is acted upon by an enzyme to produce a product) which are reacted with enzymes which are then developed on a TLC (thin layer chromatography) plate.

3. Laura describes her typical day and says, “Well, there is no typical day.” Does this appeal to you? Would you like a job where there are different things happening day-to-day or something more consistent?
   Answers will vary.

4. What is an enzyme and substrate? Research this.
   An enzyme is a protein that helps to break down or build up other molecules. A substrate is a molecule that an enzyme acts upon to create a product. An example is the enzyme lactase, which helps to break down the double sugar lactose (a sugar found in milk) into single sugars.

Click on the “**Lab Techniques**” Tab at the top of the website page. You will see some of the Ryerson University scientists at work, demonstrating lab techniques such as: gel electrophoresis, TLC and SPE (solid phase extraction).

View the TLC demonstration video. Summarize the concept in 1-2 sentences.
Summary: TLC works by separating a mixture into its individual components by their properties.

Part B: Literacy Connection

Read the June 9, 2017 CNN article "The Shifting Science of DNA in the Courtroom" by Channon Hodge:
http://www.cnn.com/2017/06/09/health/dna-technology-forensic-evidence/index.html

or

Connect to Grade 11 Genetics and Biodiversity by reading the August 28, 2014 *Science News for Students* article by Alison Pearce Stevens, "Saving the Banana."
https://www.sciencenewsforstudents.org/article/saving-banana

On the Lines: Main Idea, Who? What happened? Include 2 definitions of key words
Answers will vary.
Between the Lines: Why? Benefits? Consequences?
Answers will vary.
Beyond the Lines: Do you agree? How does this affect your community/city/country?
Answers will vary.