Title: **DNA Technology and Genomics**

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Abstract: The goal of this action proposal is to provide students the opportunity to become familiar with the processes of biotechnology and its real world application in modern day science. By using the bio-rad PV92 Informatics kit students will extract their own DNA from cheek cells, perform PCR to amplify a selected region on their DNA, and through gel electrophoresis will identify the presence or absence of a specific Alu repeat sequence of DNA base pairs in their own genome. As an enrichment activity students can further investigate the field of biotechnology through the virtual game Mission Biotech.

Rational: The mission of this activity is to introduce students to DNA technology through application of biotechnology lab techniques. Students will acquire skills through hands on lab activities and gain an understanding of the real world application of these biotechnology techniques through the sequential completion of labs from DNA extraction, amplification, electrophoresis and southern blot.

Description:

This teaching unit will be for my Advanced Placement biology students. The AP biology curriculum includes12 required labs. One of the required labs involves DNA technology such as transformation of E.coli and DNA analysis through gel electrophoresis. This lesson will apply elements of the ICORE summer institute to complete this required AP biology lab, and also to expand students' knowledge and skills on other real world research applications using biotechnology techniques.

Day 1 – CPET staff to come to Edgewater High School
I will arrange for students to be on an "in-house" fieldtrip for half of the school day. I have two classes of AP biology students, so one group of students will come to my classroom in the morning and the second group will come to my classroom in the afternoon. Both groups will be doing the same exercises.

Activity #1 – Pipetting Activity – Students will learn how to use a micro-pipette through a fun activity which will allow them to follow specific protocol to fill gridded wells to create an unknown illustration.

Activity #2 DNA Extraction Lab - Students will first extract samples of their own DNA from their cheek cells. The students will be looking to identify a specific Alu repeat (a 300 base pair repetitive sequence of DNA) within a specific region on chromosome 16. Over evolutionary time, up to 1 million copies of the Alu repeat have become randomly inserted throughout the human genome. Some individuals carry this Alu insertion and

some do not. Students will ultimately be creating their own DNA fingerprint to determine whether or not they possess this Alu repeat within their own genome.

Activity #3 – Students will then use PCR techniques to amplify their extracted DNA samples that they have collected.

DAY 2 – This lesson will last one class period.

Students will analyze samples of their own DNA (from previous day) through the process of gel electrophoresis. This lab activity will be facilitated by the CPET staff.

DAY 3 - This lesson will last one class period.

Southern Blot Simulation - A Southern blot is a method routinely used in molecular biology to check for the presence of a DNA sequence in a DNA sample. It is an alternative method to demonstrate the ability of DNA to be separated by charge and size of fragments. Students will perform the Southern Blot simulation lab to gain hands on experience on a real world biotechnology application.

Enrichment Activities:

After this series of experiments students will be able to participate in the Mission Biotech virtual gaming activity. This activity will be offered as an enrichment activity. Students will be offered extra credit to come in after school to play the game.

Student Assessment:

1. Lab reports & class discussions
2. Partner lab quiz
3. Unit assessment

ICORE Summer Institute elements specifically included:

1. Pipetting Activity
2. DNA extraction
3. PCR
4. DNA gel electrophoresis
5. Southern Blot Simulation
6. Mission Biotech Virtual Gaming

Literature Cited:

- Summer 2010 ICORE binder elements

Lab Experiment 2 – DNA extraction, PCR, and Gel Electrophoreis
Dr. Lawrence's Southern Blot Simulation Activity handout
Biorad PV92 Informatics Kit – Description

Budget and budget justification:

- Classroom set for implementation of DNA analysis labs (8 stations) $8,000-$10,000
  - P20, P200, P1000 pipettes and pipette tips
  - 1.5 ml Eppendorf tubes
  - 0.02 ml PCR tubes
  - Tube holders/racks
  - Thermal cycler
  - Analytical scale
  - Vortex
  - Mini centrifuge
  - DNA extraction reagents
  - PCR reagents
  - E-gel Powerbase
  - E-gels
  - UV trans-illuminator

- Biorad PV92 Informatics Kits (two) $ 498.50

- Visit from CPET $ 0.00

- Southern Blot Simulation $ 4.00

  - Food coloring
  - Yogurt

TITLE: DNA Technology and Genomics

SCIENCE SUBJECT: Advanced Placement Biology

GRADE AND ABILITY LEVEL: Grades 10, 11, 12 (Advanced Placement)

SCIENCE CONCEPTS: Biotechnology Lab Techniques such as:

- Use of Micropipettes
- DNA Extraction
- PCR (Polymerase Chain Reaction)
- DNA separation and analysis through DNA gel electrophoresis
- Southern Blot

OVERALL TIME ESTIMATE: Total Time: 6.5 Hours

- Half day in-house fieldtrip – 3 hours 15 mins
- Two class periods – DNA extraction/Southern Blot - 1.5 hours (2 class periods)
- Review and Discussion – 1 hour (1 ½ class periods)
- Assessment (Test) – 45 minutes (1 class period)

LESSON SUMMARY: Students will acquire skills through hands on lab activities and gain an understanding of the real world application of these biotechnology techniques through the sequential completion of labs from DNA extraction, amplification, electrophoresis and southern blot.

STUDENT LEARNING OBJECTIVES WITH STANDARDS:
The student will be able to:

1. Pipet different microliter volumes (using micropipette)
2. Extract DNA from a cell (specifically a cheek cell in this lesson)
3. Produce many copies of the target DNA (their own DNA extracted from their cheek cells) by performing a technique called Polymerase Chain Reaction (PCR)
4. Visualize their DNA bands in an agarose matrix using a DNA separating technique called Gel Electrophoresis
5. Perform a Southern Blot Simulation as a way to learn how electrophoresis-separated DNA found on the Agarose Gel can be transferred to a more permanent media such as filter membrane

STATE STANDARDS:

SC.912.N.1.1: Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following:

1. pose questions about the natural world
2. conduct systematic observations
3. examine books and other sources of information to see what is already known

4. review what is known in light of empirical evidence
5. plan investigations
6. use tools to gather, analyze, and interpret data (this includes the use of measurement in metric and other systems, and also the generation and interpretation of graphical representations of data, including data tables and graphs)
7. pose answers, explanations, or descriptions of events
8. generate explanations that explicate or describe natural phenomena (inferences)
9. use appropriate evidence and reasoning to justify these explanations to others
10. communicate results of scientific investigations
11. Evaluate the merits of the explanations produced by others

SC.912.L.16.10: Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.

MATERIALS:

Micropipette Activity:

- CPET to provide materials
- micropipettes and – one per pair of students
- trays with wells for student to pipette colored water into – one per pair students
- microfuge tubes to hold samples of colored water
- instruction sheet/chart indicating the volume of each sample that should go into the appropriate wells – one per student

DNA Extraction:

- CPET to provide materials
- bio-rad PV92 Informatics kit – one per class
- Gator aid & small cups – one per student
- Micropipettes & tips – enough per pair of students
- Centrifuge tubes
- Centrifuge –one per class
- Thermal Cycler – one per class

PCR:

- CPET to provide materials
- Materials included in the PV92 informatics kit listed above
- Distilled water
- Micropipettes and tips – one per pair of students
- PCR tubes
- Vortex – one/two per class
- Centrifuge – one per class
- Thermal Cycler – one per class

DNA GEL ELECTROPHORESIS:

- CPET to provide materials
- PCR samples from previous experiment
- Molecular weight marker – one per lab group
- Distilled water
- Micropipettes and tips – one per pair of students
- E-gel PowerBase and adapter plug- one per pair of students
- E-gel (0.8% or 1.2% with SYBR Safe) –one per pair of students
- Mini Centrifuge (0.2ml tubes) – one per class
- UV Transilluminator – one per class

SOUTHERN BLOT SIMULATION:

- Simulation kit from CPET – one kit
- Yogurt
- Water
- Instruction sheet – one per student

BACKGROUND INFORMATION:

VOCABULARY:

1. **Genetic engineering** – Process of manipulating genes and genomes
2. **Biotechnology** – is the process of manipulating organisms or their components for the purpose of making useful products
3. **Gene cloning** – is the process by which scientists can produce multiple copies of specific segments of DNA that they can then work with in the lab
4. **Restriction enzymes** – are used to cut strands of DNA at specific locations (called restriction sites). They are derived from bacteria. When a DNA molecules is cut by restriction enzymes, the result will always be a set of restriction fragments, which will have at least one single-stranded end, called a sticky end. Sticky ends can form hydrogen bonds with complementary single-stranded pieces of DNA. These unions can be sealed with the enzyme DNA ligase.
5. **Nucleic acid hybridization** – can be used to find a gene of interest among many colonies present after transformation. If at least part of the nucleotide sequence of the gen of interest is known, a probe complementary to it can be synthesized.
6. **PCR** – polymerase chain reaction is a method used to greatly amplify a particular piece of DNA without the use of cells. PCR is used to amplify DNA when the source is impure or scanty (as it would be at a crime scene).

7. **Gel electrophoresis –** is a lab technique that is used to separate macromolecules, primarily DNA and proteins, on the basis of their size and charge with the use of an electrical current
8. **Southern blotting** – combines gel electrophoresis and nucleic acid hybridization, allowing researchers to find a specific human gene. This technique is specific enough to find and note the difference between alleles. For example, it can distinguish a normal hemoglobin gene from a sickle cell gene.

Lab Techniques:

1. **Measuring volume with a micropipette** – To measure microliter volumes, a special instrument called a micropipette is used. These instruments are manufactured to deliver samples in various ranges (ex: 0.5-10 µl, 5-50 µl, 200-1000 µl, etc) and usually can be adjusted in one microliter increments. Micropipettes are the most common instruments used by molecular biologists. It is essential that they be used properly; otherwise, any work done in a molecular biology laboratory is suspect.

2. **DNA Extraction** – Cells reproduce by passing DNA from the parent cell to the offspring or daughter cell. In this activity, students will extract and observe their own DNA from their cheek cells. The cell's plasma membrane and the nuclear membrane surrounding the nucleus are made up of phospholipids. These lipids are dissolved by using a simple household detergent that contains sodium lauryl sulfate. A little table salt will also be added, which helps to eliminate the proteins, called histones, on which the DNA is wrapped. The process of removing the proteins is called "salting out". To remove as many proteins as possible, an enzyme called protease (found in meat tenderizer) will be used. The proteins and the phospholipids will form a precipitate which will settle to the bottom of the tube. The DNA is in the liquid supernatant which floats above the precipitate. Students will use filtration to separate the cellular remnants from the supernatant containing the DNA. The filtrate contains the DNA. The DNA which is soluble in water is still invisible. Students will precipitate the DNA by adding cold isopropanol inside the tube. An interface between the supernatant (water) and the alcohol will form. The DNA will precipitate out at this interface. By placing a glass rod into the interface and twisting slowly, you can "spool" the DNA onto the rod.

3. **PCR (Polymerase chain reaction)** – Produces exponentially large amounts of a specific piece of DNA from trace amounts of starting material (template). The template can be any form of double-stranded DNA. A researcher can take trace amounts of DNA from a drop of blood, a single hair follicle, a cheek cell and use PCR to generate millions of copies of a desired DNA fragment. Prior to PCR, it would have been impossible to do forensic or genetic studies with such a small

amount of DNA. The ability to amplify the precise sequence of DNA that a researcher needs to study makes PCR a very valuable tool.

4. **Gel electrophoresis** – is a lab technique that is used to separate macromolecules such as DNA on the basis of size and charge. First the DNA being studied must be cut into small fragments. This is accomplished by using restriction enzymes (derived from bacteria) to cut the DNA at specific locations, called "restriction sites". In separating DNA, the negative charges on the DNA molecules cause DNA to move toward the positive pole. The gel allows smaller molecules to move more easily than larger fragments of DNA. The DNA fragments are separated by size. Students will be looking for a specific Alu repeat (a 300 basepair repetitive sequence of DNA) on chromosome 16. During evolutionary time, up to 1 million copies of the Alu repeat have become randomly inserted throughout the human genome. Within a specific region on chromosome 16, called PV92, some of us carry an Alu insertion and some of us do not. Such variations among individuals are inherited. This is the basis of personal identification via DNA fingerprinting.

5. **Southern blotting** – is a lab technique to transfer DNA from gel to more permanent membrane. Eukaryotic DNA contains introns (noncoding sequences). VNTR - variable number tandem repeats (17-40 bases) exist at 13 sites on 12 different chromosomes. It is possible to distinguish one allele from another through southern blotting, as one allele can have a different number of VNTRs than the other.

PROCEDURE AND DISCUSSION QUESTIONS:

Day 1: Half Day (3 hours and 15 minutes)

Activity #1 – Pipetting Activity – Students will learn how to use a micro-pipette through a fun activity which will allow them to follow specific protocol to fill gridded wells to create an unknown illustration. Students will work in pairs. A brief introduction and overview of how to use a micropipette by the teacher will be discussed with the class before students will try on their own.

Discussion Questions:

1. Why must micropipettes always be used with a tip?
2. Why is plunger pushed to first stop BEFORE placing tip in the liquid to be withdrawn?
3. ____ µl = 1 ml?

Activity #2 DNA Extraction Lab - Students will first extract samples of their own DNA from their cheek cells. The students will be looking to identify a specific Alu repeat (a 300 base pair repetitive sequence of DNA) within a specific region on chromosome 16.

Prior to starting this activity, the teacher will provide background information explaining the Alu repeat. Following this background explanation, the teacher will go over each step of the DNA extraction process and discuss the purpose of each step in extracting the DNA from the cells.

Discussion Questions:

1. Explain why the soap, salt and the meat tenderizer were essential in this technique used to extract DNA.

Activity #3 PCR (Polymerase Chain Reaction) – Students will then use PCR techniques to amplify their extracted DNA samples that they have collected. Prior to starting the PCR technique, the teacher will provide background information explaining the purpose of PCR and the process. The three steps of PCR (denaturation, anneling, and extension will be discussed along with other important components of the lab such as: oligonucleotide primers, enzyme Taq DNA polymerase, and Master mix buffer.

All lab activities on Day 1 will be facilitated by the CPET staff

Discussion Questions:

1. What is PCR?

2. What are the key steps in the PCR process?

DAY 2 – This lesson will last one class period.

Students will analyze samples of their own DNA (from previous day) through the process of **gel electrophoresis**. On a previous day (before lab)…the teacher will explain "restriction enzymes" ….where they come from and what they do. Students will do a hands on activity….using scissors to simulate the activity of restriction enzymes. Once students understand what RFLPs (Restriction Fragment Length Polymorphism) are, the teacher will explain how these are used in gel electrophoresis as a discriminating tool to solve crimes scenes or study family inheritance. The students will also be directed to a gel electrophoresis virtual lab website so that they can better understand the process of electrophoresis and how it separates the DNA, and also to run through the procedure by doing the virtual lab in preparation for this day. The actual lab activity done on this day, will be facilitated by the CPET staff.

Discussion Questions:

1. What is gel electrophoresis?

2. Describe how gel electrophoresis works.

DAY 3 - This lesson will last one class period.

Southern Blot Simulation - Students will perform the Southern Blot simulation lab to gain hands on experience on a real world biotechnology application. Just prior to doing this lab, the teacher will take a few minutes to explain the purpose and procedures involved in the Southern Blot technique.

Discussion Questions:

1. What is the purpose of the Southern Blot?

ASSESSMENT SUGGESTIONS:

Objective One: Evaluate if students were able to produce correct "pattern" in their well plate after pipetting activity.

Objective Two: Students will be asked to draw a diagram of a cell. They will need to indicate which structures are involved in extracting DNA from cell…and explain how this was accomplished during the lab.

Objective Three: Students will illustrate and describe the key steps involved in PCR technique.

Objective Four: Students will draw diagram of electrophoresis gel. They will label positive and negative ends, draws bands and indicate which ones moved fastest/slowest and explain why, draw wells…and explain which way the fragments will move from the wells and why, explain what the bands on the gel represent.

Objective Five: Students will explain the usefulness of the southern blot technique.

Unit Test: Multiple Choice format to cover all objectives.

EXTENSION ACTIVITIES:
After this series of experiments students will be able to participate in the Mission Biotech virtual gaming activity. This activity will be offered as an enrichment activity. Students will be offered extra credit to come in after school to play the game.

RESOURCES/REFERENCES:

Behel, Suzy. *Principles of Biotechnology I: Laboratory Manual*. Sanford: Seminole State College, 2010. Print.

Biotechnology Explorer. Hercules: Bio-Rad, 2010. Print.

Bokor, Julie. *Biotech in the Classroom: Laboratory Manual*. Gainesville: University of Florida, 2010. Print.

Holtclaw, Fred W., and Theresa K. Holtzclaw. "Chapter 20: DNA Technology and Genomics." *AP Biology: AP Test Prep Series:*. San Francisco: Pearson Education, 2008. 134-50. Print.