Introduction to Genetics

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INTRODUCTION

This curriculum unit is intended to be used by senior students from Michael E. DeBakey High School for Health Professions (HSHP). This high school is part of the Texas Medical Center, which is the most important medical center in the world. With a student body of less than 750 students, DeBakey offers a stringent college-bound curriculum, with emphasis in science and mathematics. Also, from the worldwide diverse faculty to the multicultural student population, DeBakey is truly a global community in which excellence is the norm, and there is always room for improvement. Furthermore, DeBakey's Mission Statement is:

> To provide a challenging, well-balanced college preparatory program which focuses on educational experiences in science and the health professions and further an understanding of our multicultural community.

In addition, many DeBakey students wish to attain undergraduate degrees that prepare them for health careers ranging from medical school to graduate studies in molecular biology, biochemistry, or immunology. Recently, interest has been popularized by television and national events requiring the use of biotechnologies, such as bacterial transformation, DNA fingerprinting (e.g., CSI), paternity testing (e.g., Anna Nicole Smith), and polymerase chain reaction (PCR). As a result, this thematic unit is designed to develop a basic knowledge in the field of genetics, giving an overview of: 1) Classical Genetics, 2) Modern Genetics, 3) Molecular Biology, and 4) Population Genetics. Students finishing this thematic unit in genetics will better understand the differences and similarities between classical and non-classical genetics. Also, students will attain basic knowledge of the latest experiments and laboratory procedures which are being done in major universities, research centers, and industry. Finally, students must excel in communication, writing, presentation, and teamwork skills. All these skills are required in today's competitive workforce and educational research environments.

OBJECTIVES

The following objectives from the Texas Essential Knowledge and Skills (TEKS) will be used for this unit:

Scientific Processes

(1) The student conducts field and laboratory investigations using safe, environmentally appropriate, and ethical practices.

(2) The student uses scientific methods during field and laboratory investigations. The student is expected to:

(A) plan and implement investigative procedures including asking questions, formulating testable hypotheses, and selecting equipment and technology;
(B) collect data and make measurements with precision;
(C) organize, analyze, evaluate, make inferences, and predict trends from data; and

(D) communicate valid conclusions.
(3) The student uses critical thinking and scientific problem solving to make informed decisions.

Scientific Concepts

(1) The student knows that cells are the basic structures of all living things and have specialized parts that perform specific functions, and that viruses are different from cells and have different properties and functions.

(2) The student knows how an organism grows and how specialized cells, tissues, and organs develop.

(3) The student knows the structures and functions of nucleic acids in the mechanisms of genetics. The student is expected to:
(A) describe components of deoxyribonucleic acid (DNA), and illustrate how information for specifying the traits of an organism is carried in the DNA;
(B) explain replication, transcription, and translation using models of DNA and ribonucleic acid (RNA);
(C) identify and illustrate how changes in DNA cause mutations and evaluate the significance of these changes;
(D) compare genetic variations observed in plants and animals;
(E) compare the processes of mitosis and meiosis and their significance to sexual and asexual reproduction; and
(F) identify and analyze karyotypes.

(4) The student knows the theory of biological evolution. The student is expected to:
(A) identify evidence of change in species using fossils, DNA sequences, anatomical similarities, physiological similarities, and embryology; and

(5) The student knows metabolic processes and energy transfers that occur in living organisms. The student is expected to:
(A) compare the structures and functions of different types of biomolecules such as carbohydrates, lipids, proteins, and nucleic acids;
(B) compare the energy flow in photosynthesis to the energy flow in cellular respiration;
(C) investigate and identify the effects of enzymes on food molecules; and
(D) analyze the flow of matter and energy through different trophic levels and between organisms and the physical environment.

RATIONALE

In recent years, scientists have learned a lot about DNA from reproductive cloning (e.g., Dolly) to decoding the Human Genome. As a result, the Texas Education Agency (TEA) and AP Central are putting a tremendous emphasis on genetics for the biology curriculum, but there is no integral curriculum available as of this day that combines classical and modern genetics. This thematic unit intends to unite these intrinsic disciplines into a basic, sequential unit.

INTRODUCTION TO GENETICS

Overview and Sequence

This unit is designed to develop a basic knowledge in the field of genetics, to give an overview of: 1) Classical Genetics, 2) Modern Genetics, 3) Molecular Biology, 4) Population Genetics.

Timeline (Approximately 6 weeks)

I.	pGlo Bacterial Transformation	~5.0 hours
II.	DNA self-Extraction (Genes in a bottle)	~5.0 hours

III.	DNA fingerprinting	~5.0 hours
IV.	Techniques of DNA Analysis (PCR)	~7.5 hours

UNIT BACKGROUND

In our seminar *One Cell to Many: the Dynamics of Life*, we learned about the complex and fascinating changes that occur at the cellular level and within the entire organism. This unit will compile the main elements that gave rise to the wonderful and intriguing science of **Biology**, emphasizing genetics.

To understand the complex metamorphosis that genetics has undergone, this timeline from *Molecular Biology of a Cell* describing the major accomplishments that have reshaped and consolidated our knowledge about genetics will be given:

1869 *Miescher* first isolates DNA from white blood cells harvested from pus-soaked bandages obtained from a nearby hospital.
1944 *Avery* provides evidence that DNA, rather than protein, carries the genetic information during bacterial transformation.
1953 *Watson* and *Crick* propose the double-helix model for DNA structure based on x-ray results of Franklin and Wilkins.
1955 *Kornberg* discovers DNA polymerase, the enzyme now used to produce labeled DNA probes.
1961 *Marmur* and *Doty* discover DNA renaturation, establishing the specificity and feasibility of nucleic acid hydridization reactions.
1962 *Arber* provides the first evidence for the existence of DNA restriction nucleases, leading to their purification and use in DNA sequence characterization by Nathans and H. Smith.
1966 *Nirenberg, Ochoa,* and *Khorana* elucidate the genetic code.
1967 *Gellert* discovers DNA ligase, the enzyme used to join DNA fragments together.
1972-1973 DNA cloning techniques are developed by the laboratories of *Boyer, Cohen, Berg,* and their colleagues at Stanford University and the University of California at San Francisco.
1975 Southern develops gel-transfer hybridization for the detection of specific DNA sequences.
1975-1977 *Sanger* and *Barrell* and *Maxam* and *Gilbert* develop rapid DNA-sequencing methods.
1981-1982 *Palmiter* and *Brinster* produce transgenic mice; *Spradling* and *Rubin* produce transgenic fruit flies.
1982 GenBank, NIH's public genetic sequence database, is established at Los Alamos National Laboratory.
1985 *Mullis* and co-workers invent the polymerase chain reaction (PCR).
1987 *Capecchi* and *Smithies* introduce methods for performing targeted gene replacement in mouse embryonic stem cells.
1989 *Fields* and *Song* develop the yeast two-hybrid system for identifying and studying protein interactions
1989 *Olson* and colleagues describe sequence-tagged sites, unique stretches of DNA that are used to make physical maps of human chromosomes.
1990 *Lipman* and colleagues release BLAST, an algorithm used to search for homology between DNA and protein sequences.
1990 *Simon* and colleagues study how to efficiently use bacterial artificial chromosomes, BACs, to carry large pieces of cloned human DNA for sequencing.
1991 *Hood* and *Hunkapillar* introduce new automated DNA sequence technology.

1995 Venter and colleagues sequence the first complete genome, that of the bacterium Haemophilus influenzae.
1996 *Goffeau* and an international consortium of researchers announce the completion of the first genome sequence of a eukaryote, the yeast Saccharomyces cerevisiae.
1996-1997 *Lockhart* and colleagues and *Brown* and *DeRisi* produce DNA microarrays, which allow the simultaneous monitoring of thousands of genes.
1998 *Sulston* and *Waterston* and colleagues produce the first complete sequence of a multicellular organism, the nematode worm Caenorhabditis elegans.
2001 Consortia of researchers announce the completion of the draft human genome sequence. (Alberts 492)

Deoxyribonucleic Acid (DNA)

We can trace the discovery of the genetic role of DNA back to the beginning of the 20th century. In 1928, Frederick Griffith was studying Streptococcus pneumoniae. This bacteria causes pneumonia in mammals due to its pathogenic coat, which is where its toxicity originates. Griffith used two strains (varieties) of bacteria: a nonpathogenic (non-toxic) strain, and a virulent (disease-causing) strain. He noticed that when he injected the virulent strain after being heat-killed, mice lived, but when this strain was combined with the non-pathogenic strain the mice died (Campbell and Reece 294). Griffith referred to this permutation as transformation.

Nevertheless, he did not understand what was transforming those nonpathogenic bacteria into pathogenic ones:

> The fact that transforming activity is destroyed only by those preparations containing depolymerase for deoxyribonucleic acid and the further fact that in both instances the enzymes concerned are inactivated at the same temperature and inhibited by fluoride provides additional evidence for the belief that the active principle is a nucleic acid of the deoxyribose type. (Avery 149-150)

Once Avery had identified the transformation material as DNA, the DNA needed to be isolated, purified, and crystallized so that its structure could be characterized by X-ray crystallography. On April 25, 1953, Watson and Crick submitted a revolutionary and controversial proposal:

> We wish to put forward a radical different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis. The two chains (but not their bases) are related by a dyad perpendicular to the fibers axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine). (Watson and Crick, "Molecular Structure" 737)

Austrian biochemist Erwin Chargaff discovered two rules that helped lead to the discovery of the double helix structure of DNA ("Erwin Chargaff" *Wikipedia*). According to Chargaff's rules, Adenine will pair with Thymine and Guanine will pair with Cytosine (Campbell and Reece 296):

> The chemical formula of deoxyribonucleic acid is now well established. The molecule is a very long chain, the backbone of which consists of a regular alternation of sugar and phosphate group. To each sugar is attached a nitrogenous base, which can be of four different types. (Watson and Crick, "Molecular Structure" 737)

Considering that the students will not be able to have access to DNA crystals nor will they have X-ray diffraction crystallography technology due to the cost of equipment, students will

continue working in a middle school lab and take it to the next level. Strawberry and Banana DNA extractions are inexpensive and practical laboratory techniques to isolate DNA and engage students' interests. To demonstrate that all cells contain DNA, the "Genes in a bottle" kit (*BioRad)* will be utilized. This kit is great to illustrate DNA self-extraction, inexpensive, and easy to use. Students will extract DNA from their cheek cells. After isolation, students will be able to take home their DNA in special glass containers that are made into necklaces (Hermanson and Woodrow).

Nevertheless, to engage students with DNA activities more tangible strategies need to be used. The pGLO™ bacterial transformation kit is an excellent biotechnology laboratory that works by expressing Green Fluorescent Protein (GFP). This GFP is incorporated into a plasmid and ampicillin resistance gene, which codes for beta-lactamase (pGLO) and will be used to transform bacteria. When GFP is over-expressed the students will be able to visualize the transformed bacteria under UV light (Mardigian "pGLO Bacterial").

DNA Fingerprinting

After the isolation and characterization of the DNA molecule, DNA pervaded the field of biology, which led to the formation of new sciences, such as molecular biology, molecular genetics, and modern forensics. All of these gave rise to new experimental procedures and techniques, such as transgenic technology and DNA fingerprinting. The father of DNA fingerprinting, Alec J. Jeffrey, said:

> We tested this idea of detecting multiple hypervariable loci by hybridizing a core repeat probe to an arbitrarily chosen Southern blot carrying DNA from a family group plus a range of DNAs from various nonhuman species. Although the autoradiograph was indistinct and messy, the results were amazing—emerging from the gloom were what seemed to be highly variable profiles of DNA that looked as though they were simply inherited in the family. The penny dropped almost immediately—we had accidentally stumbled upon a DNA method with potential for individual identification. Thus, on the morning of Monday, September 10, 1984, DNA fingerprinting was born. (Jeffrey 1035)

Furthermore, DNA typing has come a long way since its accidental beginnings twenty-one years ago and has had a profound impact on individuals, society, and the law, having already directly touched the lives of millions of people worldwide. But there is much more to DNA fingerprinting than identification. Minisatellites have allowed us to identify some of the most variable and unstable loci in the human genome, and have proven superb for analyzing processes of repeat DNA instability in the human germline (Jeffrey 1035).

To understand how DNA fingerprinting works, the "Analysis of Precut Lambda DNA" *Bio-Rad™* kit will be used:

> This kit introduces students to some important principles of genetic engineering. Specifically, this exercise stresses the functions of restriction enzymes and their use as molecular biology tools when working with DNA. Using agarose gel electrophoresis, students will examine the digestion patterns, analyze the migration distances, and determine the size of the unknown DNA fragments. Although hundreds of restriction enzymes are known, for this investigation EcoR1, Pstl, and HindIII have been used to digest bacteriophage lambda DNA. Gel electrophoresis will be employed to separate the resulting DNA fragments, and nontoxic blue dye (Fast Blast™ DNA stain) will be used to stain the DNA fragments for visualization. (Mardigian "Analysis")

Polymerase Chain Reaction (PCR)

One of the most decisive moments in genetics and molecular biology was the development of the polymerase chain reaction. This new discovery revolutionized the way we practice biology today.

PCR has not only reshaped the way science is being done, but also it has affected the way we practice law and medicine. To summarize the steps of PCR by Mullis:

> Two short oligonucleotide primers are synthesized so that they are bound correctly to opposite strands of the DNA segment that will be replicated. At the points of DNA duplex formation, an added enzyme (a thermostable DNA polymerase) can start to read off the genetic code; through which two new double strands of DNA are formed. The sample is then heated, which makes the strands separate so that they are free to bind to new copies of the oligonucleotide primer and can be read off again. The procedure is then repeated time after time, doubling at each step the number of copies of the desired DNA segment. Through such repetitive cycles it is possible to obtain millions of copies of the desired DNA segment within a few hours. The procedure is very simple, requiring in theory only a test tube and some heat sources and the thermostable DNA polymerase, although commercial PCR apparatuses that manage the whole procedure automatically and with great precision. In addition, the PCR method can be used for reduplicating a segment of a DNA molecule, e.g. from a blood sample. The procedure is repeated 20-60 times, which can give millions of DNA copies in a few hours. (Mullis)

To culminate our last laboratory activity for this thematic unit in genetics, students will be performing PCR with their own DNA cells. This inquiry-based laboratory experience will encourage students to expand or clarify their knowledge of genetics. For some of them, it will even, leave a legacy that might trigger their interest in future careers in genetics, gene therapy, bioinformatics, and biotechnology. Due to the wide use of PCR technology in medicine and science, having a basic understanding of the applications and future uses is of vital importance (Mardigan "Chromosome").

LESSON PLANS

Lesson Plan One: Bacterial Transformation
Time frame: 5.0 hrs

IQ Time

Students will brainstorm examples of transformation and some of its uses

Focus

- Introduce the concept of transformation.
- Explain the concept of sterile technique
- Discuss how to work with E. coli.
- Analyze what plasmids are.
- Understand the process of transformation with a pGLO plasmid

Assessment

Knowledge:	Compare and describe the main components of transformation.
Comprehension:	Name problems that scientist will encounter using transformation.
Application:	Describe the different characteristics of a "given" transformed bacteria by structure, shape, and function.
Analysis:	Analyze 3 different plasmids that are available for bacterial transformation and identify their main applications.
Evaluation:	How would you prove or disprove the notion that transformation has pervaded biological sciences.

Guided Practice

- Teacher will introduce the concept of transformation.
- Teacher will discuss the value of plasmids in transformation and some of its uses.
- Teacher will describe the basic steps of bacterial transformation.
- Teacher will demonstrate the proper laboratory techniques for the pGLO™ bacterial transformation Kit (BioRad CAT#166-0003EDU).

Modifications

- Teacher will form groups that will balance multiple abilities.
- Teacher will pass hand-outs of the key points of the bacterial transformation lab. for the students that are having difficulties assimilating the concepts.

Independent Practice

- Students will understand the main components of bacterial transformation.
- Students will prepare Petri dishes for bacterial growth and practice safe and clean lab techniques.
- Students will insert the pGLO plasmid and analyze the results of this lab.
- Students will do a comparison of all the pGLO results and explain possible error analysis.
- Students will analyze proliferation of colonies, interaction between genes and environment, and calculate transformation efficiency.
- Students will present their experimental results and conclusions, and discuss experimental designs and future applications.

ELT (Extended Learning Time)

Students will write an essay about the significance of the transformation in biological sciences.

Lesson Plan Two: Self-Extraction of DNA Analysis
Time frame: 5 hrs

IQ Time

Students will brainstorm applications for DNA extraction.

Focus

- Introduce the concept of DNA extraction
- Explain the concept of sterile technique
- Unique opportunity for self DNA extraction
- Exposure to DNA technology
- Understand the properties of DNA and its applications

Assessment

Knowledge:	Describe the main components of DNA extraction.
Comprehension:	Name problems that scientist will encounter using DNA.
Application:	Describe the different characteristics of a DNA molecule by structure, shape, and function.
Analysis:	Analyze DNA Replication, Protein Synthesis, and Genetic mutations.
Evaluation:	How would you prove or disprove the notion that genetic mutations have transformed life?

Guided Practice

- Teacher will introduce the concept of DNA extraction.
- Teacher will discuss the value of DNA extraction and some of its applications
- Teacher will describe the basic steps of DNA self-extraction.
- Teacher will demonstrate the proper laboratory techniques for the Genes in a Bottle Kit (BioRad CAT#166-2200EDU)

Modifications

- Teacher will form groups that will balance multiple abilities.
- Teacher will pass hand-outs of the key points of the DNA self-extraction lab for the students that are having difficulties assimilating the concepts.

Independent Practice

- Students will recognize the main components of DNA.
- Students will extract some cheek cells for DNA isolation and practice safe and clean lab techniques.
- Students will transfer their own DNA into a necklace and analyze the results of this lab.
- Students will present their experimental results and conclusions, and discuss cloning, transgenic technology, introduction of DNA profiling, and future applications.

ELT (Extended Learning Time)

Students will write an essay about the importance of DNA in biological sciences.

Lesson Plan Three: DNA Fingerprinting Analysis
Time frame: 5 hrs

IQ Time

Students will brainstorm why monozygotic twins have the same DNA.

Focus

- Understand the use of restriction enzymes as biotechnology tools
- Become familiar with principles and techniques of agarose gel electrophoresis
- Generate a standard curve from a series of DNA size standards
- Estimate DNA fragments sizes from agarose gel data

Assessment

Knowledge:	Define the main components of DNA fingerprinting.
Comprehension:	Name problems that scientist will encounter dealing with DNA fingerprinting.
Application:	Describe different characteristics of restriction enzymes by structure, shape, and function.
Analysis:	Analyze a gene map and create a new one with specific restriction sites.
Evaluation:	How would you solve a crime case with DNA fingerprinting analysis?

Guided Practice

- Teacher will introduce the concept of DNA fingerprinting.
- Teacher will discuss the value of restriction enzymes in research and some of its applications.
- Teacher will describe the basic steps of DNA fingerprinting.

- Teacher will demonstrate the proper laboratory techniques for the Analysis of Precut Lambda Kit (BioRad CAT#166-0001EDU).

Modifications

- Teacher will form groups that will balance multiple abilities.
- Teacher will pass hand-outs of the key points of the DNA fingerprinting lab for the students that are having difficulties assimilating the concepts.

Independent Practice

- Students will understand how restriction enzymes operate.
- Students will pour their agarose gels for gel electrophoresis to separate DNA bands that are cut by restriction enzymes, and practice safe and clean lab techniques.
- Students will analyze their completed agarose gel to interpret and draw conclusions for this lab.
- Students will present their experimental results and conclusions, and discuss ~~about~~ future applications of DNA fingerprinting and restriction enzymes.

ELT (Extended Learning Time)

Students will write an essay about the major uses of DNA fingerprinting.

Lesson Plan Four: Polymerase Chain Reaction
Time frame: 7.5 hrs

IQ Time

Students will write a one page short story of a "given" crime that caught their attention.

Focus

- Analyze the uses of DNA fingerprinting
- Explain DNA testing
- Common characteristics of wrongful incarceration due to DNA mistesting
- Analyze other applications for DNA testing

Assessment

Knowledge:	Compare and describe the main components of DNA testing.
Comprehension:	Name problems that forensic science will encounter in delineate DNA testing criteria.
Application:	Describe, as if you were an agency, the uses of DNA testing.
Analysis:	Analyze the classification system utilized to categorize DNA patterns.
Evaluation:	How would you prove or disprove the notion that everyone has elements of identification and individualization in a 'given' crime.

Guided Practice

- Teacher will introduce the concept of DNA sequences, in particular the dimorphic Alu sequence.
- Teacher will discuss the value of population genetics in research and some of its applications.
- Teacher will describe the basic steps of polymerase chain reaction.
- Teacher will demonstrate the proper laboratory techniques for PV92 PCR Informatics Kit (BioRad CAT#166-2100EDU).

Modifications

- Teacher will form groups that will balance multiple abilities.
- Teacher will pass hand-outs of the key points of the PV92 PCR lab for the students who are having difficulties assimilating the concepts.

Independent Practice

- Students will comprehend the basis of polymerase chain reaction (PCR).
- Students will pour their agarose gels for gel electrophoresis to separate DNA bands that are cut by restriction enzymes, and practice safe and clean lab techniques.
- Students will analyze their completed agarose gel to interpret and draw conclusions for this lab.
- Students will present their experimental results and interpret those results with population genetics to draw conclusions, and discuss future applications of PCR.

ELT (Extended Learning Time)

Students will write an entry in their journals to describe the significance of PCR in law, medicine, and biomedical sciences.

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