YALE NATIONAL INITIATIVE
to strengthen teaching in public schools®

Curriculum Units by Fellows of the National Initiative
2013 Volume VI: Genetic Engineering and Human Health

DNA in Forensic Science: genetic engineering applications in forensics

Curriculum Unit 13.06.07, published September 2013
by Vanessa Vitug

Introduction & Background

Genetic identification, paternity testing, genetically modified foods, and gene therapy are some of the applications in which genetic engineering is widely used today. It seems that you can't turn on the television without a CSI (Crime Scene Investigation) or Maury Povich show asking for the results of a DNA test. The field of genetics has gone beyond simply mapping the sequence of human DNA. New frontiers in biotechnology, genomics, and biomedical engineering are driving a debatable question, "How much information is too much information." But, what is DNA? Does the average person or student really understand the science of the DNA tests they hear about? If you had the ability to predict the diseases or conditions you may have, would you want to know? How important is it to know everything? These are some questions that can be explored through study of genetics and genetic engineering.

The last 50 years have seen an explosion of scientific and technological advancement in DNA technology. This is especially true in the field of DNA forensics. The application of DNA testing in crime cases began in 1984 with Alec Jeffreys' work on DNA fingerprinting. [1] Today, the Innocence Project states that nationwide 309 post-conviction DNA exonerations have occurred showing yet another use of DNA beyond its gene therapy potential. [2] Furthermore, many of the victims of 9/11 were identified using DNA identification technology. How were scientists able to do this?

With the prevalence of DNA in the media, my students naturally have a fascination for its applications in the real world. Thus, in my forensics science course, I hope to review basic DNA biology and genetics. It will serve as a starting point for the introduction of a routinely used genetic engineering tool for DNA amplification. Finally, students will learn about DNA profiling and apply their acquired skills in laboratory activities. Over the course of four weeks, students will learn the skills needed to run their own PCR (Polymerase Chain Reaction) centered on a mock-forensic case.

Mt. Pleasant High School in East Side San Jose California had a student enrollment of 1556 in the 2012-2013 school year. In the past four years, student enrollment has dropped from 1927 students in 2008. [3] We have the added task of competing for enrollment against newly formed charter schools and the movement of families out of the area due to the high cost of living. This trend has meant that Mt. Pleasant High School has

been finding new ways to retain its student population, while continuing to serve its primarily Hispanic/Latino student body. Mt. Pleasant continues to be in Program Improvement, with the 2012 school being our third year. Within our largest population, Hispanic/Latino students, 800 of 1085 are considered Socio-economically disadvantaged, 581 are considered Limited English Proficient, and 176 are considered Special Education eligible. [4] Furthermore, according to No Child Left Behind our school has not met its AYP (Adequate Yearly Progress) for several reasons. One reason is that our graduation rate for 2012 was 76.2%, far from the target goal of 90%. The second reason for not meeting the AYP is that our English and Mathematics proficiency rates for all subgroups were nearly 20% below the target of 77.8% and 77.4% respectively. [5] Though some improvements have been made, overall our school strives to increase success among our EL (English Learner) students in order to increase our API scores and meet AYP.

With the added challenge of Common Core State Standards we are seeking new ways to incorporate real-world applications into our classrooms. In examining how we currently teach our classes, it is clear that we can no longer just teach one subject. High school courses are being taught with an interdisciplinary eye. Math classes must move towards incorporating reading and problem solving skills beyond manipulation of numbers. English classes must incorporate history and science readings beyond literature and writing. Science in turn must teach writing, math, in the context of the subject matter being taught. Thus, as we move towards infusing our everyday lessons with interdisciplinary material we hope students will be able to articulate their understanding not only in the classroom but during the state tests.

Rationale

Forensic Science is a new course at Mt. Pleasant High School. Students taking Forensics are usually in the 11 th /12 th grade having successful completed Biology, Chemistry, Algebra 1, and Geometry. Most students entering Forensic Science are concurrently enrolled in Algebra 2 or Math Analysis. Students have prior knowledge of algebraic conventions and probability, which they will need for the course. Forensic Science is broken into two semesters; one semester focuses on the biological science aspects of forensic science and the other on physical science. Many students who take forensics are interested in pursuing careers related to criminal justice, medical forensics, or administration of justice. Throughout the year students learn about DNA fingerprinting, trace evidence, ballistics, toxicology, chromatography, blood splatter analysis, odontology, skeletal dimorphism, and fingerprinting. The course, though new, is gaining popularity frankly because of shows like CSI and the number of labs our students take part in. They enjoy the idea of solving cases and learning using a hands-on approach.

Because weekly laboratory work is new to many of my students, we strive to instill laboratory skills and laboratory writing throughout the semester. With Common Core State Standards and the Next Generation Science Standards around the corner, we are making strides to help our students understand the application of the content taught. Thus, Forensics Science is an ideal course to add to our current class offerings.

Challenges do exist within our new course. One major challenge is our students' unfamiliarity with the laboratory equipment. Students studying forensic science have never used the majority of the equipment we use in lab. These include the use of gel electrophoresis equipment, micropipettes, heat blocks, water baths, and thermal cycler, which will be used in this unit. Thus, lessons in basic laboratory skills need to be

incorporated as students learn the purpose behind the protocols they will follow in lab. Another issue we struggle with is the lack of depth in student's biology preparation. In biology, students are introduced to DNA structure, Mendelian genetics, and the Central Dogma, but beyond paper labs on transcription and translation, students do not get a chance to actually apply their learning in the laboratory or as it relates to the real world. Thus, my intended unit will strive to aid my students in reviewing the material they have already learned and extend it into the actual real-world applications of DNA. The material will be taught over a course of four weeks of fall quarter, but will be revisited throughout the semester.

DNA Biology

The history of DNA (Deoxyribonucleic Acid) began in the mid 19 th century with Friedrich Miescher's discovery of a substance inside the nucleus. Not until 1919, was the name "nucleotide" coined when Phoebus Levene for a molecule containing deoxyribose sugar, phosphate, and various bases. Once Rosalind Franklin's x-ray diffraction images were seen by James Watson and Francis Crick, the structure of DNA was soon discovered. In 1953, Watson and Crick revealed that DNA is a coiled double helix composed of two complementary strands held together by hydrogen bonds. [6] A single strand of DNA is a long polymer of nucleotide units. The four bases in DNA are purines (guanine and adenine) and pyrimidines (thymine and cytosine). The two strands of DNA are complementary but antiparallel running 5' to 3' direction and its complement running from 3' to 5'. [7]

In the human cell, DNA carries instructions for all cellular functions. Humans have about 50 trillion cells. Each cell has a set of 23 chromosomes tightly coiled inside the nucleus of cells. [8] As humans grow or as cells need replacement they undergo the process of mitotic division to proliferate or replace cells that have aged or are damaged. This process is achieved through mitotic division. In mitosis, a mature cell undergoes changes which allow it to replicate (make exact copies) of its DNA: the two copies are separated into two cells, which each contain an exact copy of the original DNA. The process of replication is important because it allows the cell to maintain a diploid state (having a set of chromosome inherited from their parents), and allows all cells in the body to contain the exact same DNA. All cells except those destined to be sperm or egg cell undergo mitotic division since sex cells contain half the amount of chromosomes. Thus 22 out of 23 are called autosomes and the last is the sex chromosome since they determine a person's gender.

In a strand of DNA there are approximately 3 billion bases. Of these bases are segments of DNA which specifies codes for protein synthesis called genes. There are ~25, 000 genes coding for various cellular functions (i.e. enzymatic activity) and traits like eye color. Each gene is its own instruction set and the length of a gene varies depending on the number of base pairs. Within a gene segment are control regions for activation and deactivation of the gene, short start and stop codes for control of protein synthesis, and the specific sequence that encodes the sequence of specific amino acids in the protein. [9]

Transcription and Translation

The process of making proteins is an essential topic covered in all Biology classes. This is due to the importance of proteins for cell structure, function, and regulation. In order to make new proteins, 20 amino acids combine in a specific order to create long chains of unique protein products. How they are arranged into amino acids chains are determined by their original gene code. The "Central Dogma" of Biology describes this

process. The Central Dogma states that DNA is transcribed into RNA and RNA is translated into Proteins. [10]

During transcription, the DNA gene segment is transcribed into mRNA (messenger RNA). Transcription begins with the unzipping of the double helix. An enzyme RNA polymerase binds to specific starting segments of the single stranded DNA called a promoter region. The single strand of DNA is transcribed into mRNA, where thymine is replaced by a new base - uracil. Uracil binds to its complement adenine. Once a terminator sequence is reached by the RNA polymerase, the mRNA strand is released from the DNA, completing the transcription process. [11]

When mRNA leaves the nucleus for the cytoplasm of the cell, the process of translation begins. Segments of the mRNA not needed for protein synthesis (introns) are excised, leaving the coding "exons" regions available to complete the synthesis of proteins. Free amino acids within the cytoplasm are linked together inside a ribosome, using the mRNA template. The ribosome serves as the cell factory through which the new polypeptide (a chain of amino acids) is formed. This process occurs in three steps: Initiation, Elongation, and Termination. During Initiation, small ribosomal units with tRNA (transfer RNA) bounded to a methionine amino acid begins searching for the start sequence on the mRNA strand. Once found, the large ribosomal unit binds to complete the ribosome and protein synthesis proceeds. In the second step, Elongation, new tRNAs with new amino acids sequences (anticodons) attach to its corresponding set of three nucleotide sequence (codons) on the mRNA. As more anticodons match to its codons, chains of polypeptide are added, causing the polypeptide to elongate. A DNA codon table shows exactly which anticodon-amino acid corresponds to a specific codon. This same codon table also shows the sequence for start and stop codons. Once a stop codon is reached, the fully elongated polypeptide or protein chain is released and the ribosome unbinds from the mRNA strand completing the Termination stage of translation. Though the protein is complete, it may require additional factors or steps (called post-translational modifications) to activate the protein for its purpose. [12]

DNA in Forensic Science

Today, approximately half of all criminal cases involve DNA testing. Cases involving paternity, sexual and violent crimes, bioterrorism, disease causing pathogens, and even illegal hunting can involve DNA tests. [13] It is well known that DNA makes each person unique. But how and what characteristic of DNA distinguishes one person from another? If twins are essentially identical, what can be used to distinguish them? To answer these questions, an understanding of protein alleles, polymorphisms of DNA, and probability is necessary.

Of the entire DNA in humans approximately 99% are shared between all other humans, leaving our "uniqueness" to occupy 1% of our entire DNA. [14] To grasp this concept we will need to understand how and where our DNA is unique. For example, there are many variations of the gene for eye color, each variation is called an allele. The terms heterozygous and homozygous describe whether an individual carries two similar pairs of an allele or two different pairs. Alleles come in pairs because genes are inherited, thus one allele is maternally inherited and the other paternally inherited. The actual genotype (genomic makeup) can be determined using Mendel's laws of inheritance. How the genotype is expressed is known as the phenotype. The mixing of genotypes from two parents to a child contributes to the uniqueness of an individual.

Restriction Enzymes

Knowing the genotype for an allele and its possible phenotype and location (locus) has many uses: gene therapy, genetic analysis, and DNA Profiling. A person's DNA profile or DNA makeup can be analyzed with restriction maps. Restriction maps are created from fragments of DNA which have been cut by restriction enzymes. We refer to restriction enzymes as molecular scissors since they are able to recognize specific segment of bases on a strand of DNA. For example, EcoRI is a restriction enzyme that recognizes the sequence 5' – GAATTC- 3'. When the strand is cut between the guanine (G) and the adenine (A), the result is a sticky end DNA fragment. Other restriction enzymes cut DNA in a blunt fashion, with no single strands of bases sticking out. Having some knowledge of restriction enzymes and their target genetic sequence allows us to use probability to predict how long a fragment may be. Since there are only 4 bases, we can predict that the same genetic sequence will occur at every 4^n base pair, where n is the number of bases recognized. For EcoRI, which recognizes the hexanucleotide of GAATTC, the same sequence would appear every 4^6 or 4096 bp. D. Nicholl in *Genetic Engineering* states, "fragment length is dependent on the frequency of occurrence of the recognition sequence." [15] The use of restriction enzymes led to the beginning of a genetic fingerprinting since restriction maps provided a visual representation of a person's genetic makeup, in the form of the lengths of fragments that are produced with a particular restriction enzyme (or combination of enzymes). When comparing two samples of DNA, fragments from two different unrelated persons should show a banding pattern that was different or non-overlapping. On the other hand, two people who are related may show an abundant amount of overlap between their DNA fragment lengths, reflecting similarilities in the base sequences in the DNA. Knowing DNA can be fragmented and targeted paved the way for other studies using restriction maps.

Polymorphisms

With the completion of the Human Genome Project in 2003 geneticists were able to retrieve the exact sequence of human DNA. The location of alleles became known. With this information variations of the gene could be mapped out. Variations in a gene, or polymorphisms, are categorized into two subgroups: Single base polymorphism and length polymorphism. [16] In a single base polymorphism one complementary pair of bases is replaced with another (i.e. G-C would be replaced with A-T). In the second type or polymorphism, called length polymorphism, a gene sequence has short repeated sequences which extend the length of the strand. For example, a section of DNA may have a region with TAT, but its other form may have 3, 4, 5 repeated TAT in a row rather than one. This in effect lengthens the sequence. [17]

Repeat units of DNA called satellite DNA vary in length from several hundred to several thousand bases in the repeat. Minisatellites or variable number of tandem repeats (VNTRs) are about 10-100 base pairs long. Those that are smaller in length, 2-6 bp, are called microsatellite or short tandem repeats. STRs are a type of length polymorphism that appears in tandem or next to each other. The length of many STRs is only 2-5 bp long but repeated many times in a row. The number of repeats can be distinctive for an individual: using several satellites allows forensic analyst to decrease the chances that a set of STRs belong to someone other than the source of the sample. Thus, the number of STRs and its location on the chromosome contribute to the "uniqueness" of an individual. [18]

Though STR loci mapping is the most widely used technique in forensic DNA testing, there is also an emerging method that has advantages over traditional STR analysis. SNP (Single Nucleotide Polymorphism) genotyping makes use of the abundance of SNPs in the human genome, which is close to 5 million. According to Butler, Coble, and Valone in their paper for *Forensic Science Medical Pathology* there are several advantages with

SNP genotyping. An advantage with SNP use in forensic science is that minute amounts of DNA are needed for SNP analysis. Also highly degraded samples can be analyzed as long as they are amplified. More importantly, a smaller region can be detected since SNPs markers can detect a single nucleotide versus STRs which require many nucleotides. SNPs, unlike STRs, have a relatively low rate of mutation as compared to STRs which have 100 times greater mutation rate. [19] However, despite the advantages of SNPs, the use of a STR profile in the forensics analysis will not be replaced soon. Butler, Coble, and Valone contend that STRs will continue to be used in the field because an STR profile requires only 13-15 STRs instead of the 40-60 SNPs needed to provide STRs statistical power of a unique match. [20]

In recent years, the US has collected and stored DNA information to create DNA banks. One such database is the FBI's CODIS (Combined DNA Index System). CODIS focuses on 13 core STR loci that are used for DNA analysis. Law enforcement officers can compare their samples with known reference samples from CODIS when they lack a suspect. With more profiles added to CODIS many cases, recent and cold, have a higher chance of being solved. Today, many states collect the DNA information from those convicted of violent and non-violent crimes. [21]

Despite advances in PCR (Polymerase Chain Reaction) technology, DNA collection and handling protocols, errors can arise. To reduce DNA mismatch, experts rarely state that there is a 100% DNA match, often they use the term Random Match Probability (RMP) to describe the uniqueness of DNA profile. This number makes use of the product rule of probability to state the chance of finding the genetic markers within the DNA profile in a target population. The more genetic markers available, the RMP becomes smaller and smaller, increasing the likelihood that the profile is unique to its source. [22]

Polymerase Chain Reaction

In many criminal cases, a relatively small amount of DNA is collected at the scene of the crime, thus methods for amplifying or making many copies of the collected DNA is necessary for effective analysis. Before the process of Polymerase Chain Reaction (PCR) was invented by Kary Mullis in 1983, methods for amplifying a genetic sequence were timely and labor intensive. Thus, the discovery of the PCR allowed for a faster method of amplifying minute quantities of a DNA sample, which has revolutionized crime scene analysis. The PCR process includes a series of cycles in which the purified DNA sample is denatured, annealed with primers, and extended at the targeted sequence. After each cycle, the newly synthesized copies, called amplicons, become the template for the next cycle. Thus, within a few short hours millions of copies of the original small sample of DNA are available for better viewing or for storing. The amount can be quantified by the formula 2^n where n = the number of cycles. [23] An advantage of PCR over other methods of amplification is that very little DNA is needed (~ 5-100µl), though most protocols for PCR ask for ~ 20-50µl. [24] Today, the availability of PCR kits has allowed for forensic scientists to simply add their DNA sample for amplification.

Though commercial kits allow laboratories to simplify the preparation of targeted DNA, students need to first be familiar with the idea of targeting a sequence with restriction enzymes. A discussion of each step of the polymerase chain reaction is will follow a lesson in restriction enzymes. My students can follow laboratory protocols as long as they are able to practice the skills repeatedly, however when asked to verbalize their understanding of "why" they are doing what they are doing a gap in their knowledge becomes evident. Before

students are allowed to purify their DNA samples, and run them through the thermal cycler, they will need to understand DNA extraction, denaturation of DNA, annealing of primers, and extension with nucleotides. In the end, students will have to predict the expected results of the gel electrophoresis of their amplified samples.

DNA extraction

Before DNA is amplified in a thermal cycler, the DNA must be extracted from the collected sample. To amplify a sample of DNA that has been collected from a crime scene several steps need to be taken to separate DNA from the cell. In essence DNA extraction means the cell membrane and nucleus need to be broken to expose the DNA. The trick is to break the cell's membranes without destroying the DNA. This can be accomplished through several different extraction methods. The use of Chelex beads will be discussed since that method will be used in my forensic science class.

Chelex, created by the Bio-Rad company, is a resin that is added to a sample of DNA. In effect Chelex adds magnesium ions which deactivate DNA nuclease which would otherwise digest the DNA strand for re-use as free nucleotides. [25] Before the addition of Chelex, heat is applied to the sample to open the cells and separate the bonded strands. Chelex resins are negatively charged and help to remove positive metal ions. In order to prevent DNA nucleases from becoming activated, Chelex resins bind Mg+ ions thus preventing the nucleases from being activated. [26] In doing so, nucleases are prevented from degrading the DNA strand. After the sample is centrifuged, purified DNA can be removed from the supernatant since the Chelex resin is forced to the bottom of the centrifuge tubes during centrifugation.

Other methods of extracting DNA include the use of organic chemicals (phenol-chloroform), specialized cellulose paper called FTA™, and differential extraction. All achieve the same purpose; however the Chelex method for DNA extraction is ideal for the classroom, because it does not require hazardous chemicals like the organic method. FTA™ paper is ideal for running multiple samples on an automated robotic workstation and once processed samples can be stored for multiple years. [27]

The thermal cycler is an important tool in performing PCR analysis. The thermal cycler performs the series of heating and cooling steps that allows DNA to denature, anneal, and extend with each cycle. Typically PCR amplification in thermal cycler will run for 25-30 cycles.

Denaturation of DNA

Recalling that hydrogen bonds hold DNA strands together, this bond is easily broken. Increase in heat will break the weak hydrogen bond causing the double helix to open or denature. Denaturation of DNA requires the sample to be heated above 90 ? C. This does not break the bonds between the phosphate and deoxyribose sugar because they are covalently bonded, but interrupts the hydrogen bonds.

Annealing of Primers

The annealing process is the second step in PCR. Addition of primers "activates" free nucleotides to begin the DNA polymerase-mediated extension process. [28] When they bind to DNA, primers act as signals, or starting points, for the action of DNA polymerase. One primer will bind upstream and the second downstream thus flanking the DNA strand. The primers are added in a 5' to 3' direction and the free nucleotides are added in the same manner. This annealing process occurs at a lower temperature, usually between 40 ? C to 65 ? C.

Commercially purchased PCR kits contain primers that serve to target the genetic sequence to be amplified.

Primers are synthetically engineered and specifically designed to amplify a particular genetic sequence. Today many companies manufacture primers that are designed for a specific sequence. Scientists and laboratories can find catalogs on the World Wide Web for their target sequence. Primers are listed according to their genetic sequence, allowing scientist to choose the appropriate primer for their PCR samples.

Elongation

Addition of free nucleotides is controlled by the presence of a DNA polymerase that is thermally stable. [29]*Taq* polymerase is a commonly used polymerase because its bacterium of origin (*Thermus aquaticus*) is a thermophilic organism found in hot springs.

The advantages of PCR include the relatively short amount of time it takes to amplify a specific gene of interest. Another is that a small sample of DNA is needed (as little as a single cell!), and crude preparations such as blood, semen, or saliva can be processed. On the other hand, PCR's limitations include false positives due to contamination with DNA from those handling the samples. It is also limited by the targeted gene sequence it is amplifying.

Conceptually PCR is easy to understand, but there are things that can go wrong in the process. Preparation of the PCR samples involves pipetting small quantities of DNA, primer, master mix, and purified DNA, which can lead to errors, either in the quantity moved from one tube to another or in contamination between samples. PCR tubes are tiny, holding only .2 ml. When exposed to open air, there is a risk of the sample evaporating, since it is often only ~ 20-50µl. Another problem that may arise is the creation of primer dimers. This occurs when primers bind before amplification. This premature binding of primers results in primer dimers which tend to be amplified more than the target sequence since they are only a few nucleotides in length as compared to the length of target sequence.

To prove that a DNA sample is being amplified and the "master mix" of primers, nucleotides and polymerase are all working, positive and negative controls are amplified along with the samples. Once complete, samples are loaded into gel boxes for electrophoretic separation.

Gel Electrophoresis

Gel electrophoresis is a tool that separates different lengths of DNA. In forensics it is a routinely used in DNA Typing, drug analysis, explosive residues, gunshot residues, and forgery analysis. Because DNA's backbone contains many phosphate groups, fragments are negatively charged. This occurs because at neutral pH the phosphates in DNA readily give up their hydrogen ions to become negatively charged. When placed in a gel and subjected to an electric field DNA will migrate towards the positive anode and move away from the negative cathode.

Agarose is a commonly used material for making gel slabs. Agarose is a large polymer of sugar found in marine algae. The powdered seaweed is about 200nm in diameter. When mixed with a buffer solution, heated, and allowed to cool, DNA samples can be loaded into small wells. Each tiny well positioned at the cathode end of the gel box can hold between ~ 5-10µl of DNA. Submerged in buffer of Tris-Acetate (TAE) or Tris-Borate (TBE), the samples move towards the positive anode, at rates that depend on the length of their strands. The

electrical field can be controlled by a power supply which can vary the voltage running to the electrode on each end of the gel box. Typically, a 10 x 40 cm gel requires 100-600 v to separate the DNA strands. [30]

Because DNA fragments will move at speeds defined by their size (length), small fragments migrate at a faster rate than long fragments, thus at the end of gel run the DNA sample that was loaded into the gel well is separated into bands. If a DNA ladder has been run along with the sample, the relative length of each band can be measured. DNA ladder provide known fragments to compare samples against. An example of a DNA ladder is Lambda phage DNA. Lambda phage is digested using restriction enzymes, its resultant fragments are identify and thus later used as a genetic marker or DNA ladder. To view the fragment after its run dyes are loaded either before or after the run to enhance the viewing of the DNA fragments.

In order to visualize the banding pattern of DNA on a gel, dyes are often added to the gel. One type of commonly used dye is a fluorescent dye called Ethidium Bromide. EtBr binds between the bases of the DNA. When viewed under an ultraviolet illuminator, the fluorescent dye can be visualized and photographed. The drawback of using EtBr is that the chemical is carcinogenic and thus must be carefully handled.

Other gels made of polyacrylimide are available and are different than agarose gels because of their pore size. Polyacrylamide gels have a pore size of ~100-200 Å versus agarose whose pore size runs between ~1500 - 2000 Å. [31] In a high school laboratory class, agarose serves the purpose better because gel powders are relatively inexpensive.

Another electrophoresis method is capillary electrophoresis (CE). Capillary electrophoresis instruments are now routinely used in modern forensic DNA analyses. The advantages of CE are that it requires minute amounts of DNA, a result is available within minutes rather than hours, there are no gel slabs to cast and resolve, and the process is fully automated. CE has another major advantage because a sample can be separated with a single base difference. CE results are available electronically, making the photographing gel samples unnecessary. This printout or visual of a CE result is called an electropherogram. [32]

Strategies and Classroom Activities

Forensic Science at Mt. Pleasant High School is structured around a case every six weeks. Students are in small cooperative laboratory groups and act as a forensic team. Within their cooperative learning group, students perform all laboratory assignments as a team thus allowing for shared responsibility and accountability. Each quarter has a focus whether it is DNA in Forensics, Ballistics, Trace Evidence, or Skeletal Remain Identification. My unit will be taught during the "background" portion of the first six weeks of school. During this time, students focus their readings, lectures, activities, and labs towards the topic of DNA in forensics, the remaining two weeks will be spent analyzing a mock crime case which will require students to apply their previous learning.

Since many of my students have never kept a science journal or laboratory notebook we begin the school year with guidelines for a student laboratory journal. Journaling or laboratory writing is a skill students develop through the course. This journal is similar to one a scientist keeps as they are doing their research, but is designed to help students understand the value of being meticulous when collecting data, analyzing data, and making conclusions. Initially my students are reluctant to practice their note-taking in the notebook. But, as

they insert information relevant to the mock cases they are trying to solve, it becomes a vital tool in the laboratory. Students need instruction in structure, thus, we (forensic teachers) give them a very detailed description/ instruction for their laboratory notebook. (Appendix 2)

Common Core Standards are slowly being adopted into our school. Having students keep a journal in which they already write their claims, design experiments to research their claims, then evaluate the results addresses the Writing Standards for Literacy in Science. (Appendix 1) Students will need to demonstrate and express their understanding of the content using their laboratory notebook. In this manner, they are held responsible for taking Cornell Notes on readings and lecture, and reflecting on their level of understanding. More importantly, the journal is for laboratory protocol. It is meant to be a students' guide to laboratory practices and experiments. Students will need to keep detailed notes on the components of this unit.

Initially students will insert protocols for DNA extraction, followed by PCR, then Gel Electrophoresis. However, the guidelines they are given are bare-bones and will require students to insert values, drawings, notes, and modifications as necessary. In the end, all students are required to reflect on the labs discussing results, conclusions, cause of errors, reasons for modifications and more importantly relevancy to the background content taught. At the end of each lab, student's notebooks will be signed off by their instructor for completion and for comments.

Activity 1 – DNA Puzzle & Web Assignment

My unit will begin with a review of the DNA Biology. Students have made models of DNA Structure in the 9 th grade, thus in the 11 th & 12 grade, they are asked to review their understanding through a jigsaw puzzle of DNA's components. The puzzle allows kinesthetic and visual learners an opportunity to reacquaint themselves with the chemical structure of DNA. The DNA puzzle allows students to recall the components of a nucleotide and the complementary base pairing of adenine to thymine, guanine to cytosine. In addition, this manipulative allows for guided classroom instruction which will include the following questions:

Questions for DNA Puzzle:

1. What are the components of DNA?

2. Do you see a pattern in its structure?

3. Describe the pairing of the nitrogenous bases.

4. What happens when a base is mis-paired?

5. How does RNA differ from DNA?

6. Simulate replication of DNA by using the puzzle pieces from your group.

Once complete, students should be able to verbalize their understanding. As a form of informal assessment, students will be asked to answer one question regarding the day's topic as they leave the class. This method of informal assessment has many names, but is easily recognized as an "exit ticket."

Through a web assignment (Appendix 4) students will be able to engage in a virtual laboratory setting in preparation for the actual lab. The web assignment is meant as a follow-up to the puzzle piece activity but also an introductory discussion into PCR. The assignment makes use of several videos and a virtual lab from

various websites. Web-based-assignments are regularly included as part of the class since students are so adept at using the web for their learning. Though computer access is sometimes a challenge, students' needs are met by having flexible schedules and turn-in options for an assignment that requires technology. Once students have completed the assignment, lecture/discussion follows to answer lingering questions and to assess their general knowledge regarding the topic before the lab actual lab activity.

Activity 2 – Laboratory Skill: Micropipette Use

It should be noted that many students have not had any familiarity with laboratory equipment beyond microscopes and slides. Therefore, it is necessary for students to practice with the equipment. An activity we will do once the concept of the unit is understood involves the proper use of micropipettes. Students will "Find the message" using a set of micropipette instructions. A simple grid marked with A-F on the x-axis and 1-12 on the y-axis can be used for a practice. Microplate wells work well for this activity since they can be re-used. Students follow the list of volumes & locations to be transferred from a small centrifuge tube onto their grid. If students have followed the instruction set correctly, they should have a colorful message of different volumes that may say DNA or PCR as shown in Appendix 5. [33] Similar activities will also be taught regarding appropriate use and care for gel apparatus and thermal cycler.

To practice students' problem solving and gel electrophoresis skills, a Mock-Paternity Lab will follow micropipette and gel apparatus introduction. The Mock-Paternity Lab is a culminating activity for students after viewing Learn Genetics Gel electrophoresis animation video. [34] In the paternity lab, different dyes of known banding patterns are used to simulate a child, a mother, and potential fathers. Having been given samples of all possible "father" candidates, the students will need to decide if the child is the biological child claimed by the mother. Thus, students will have to assert their claims and support them with photographic evidence from their gel results.

Activity 3 – Mock-Crime Case

Besides being able to follow a laboratory protocol for DNA extraction, gel electrophoresis, and PCR students will be assessed both in writing and orally once their mock-case review is completed at the end of the six weeks (Appendix 3). Having completed the practice labs, students will be given a case summary, supporting documents, and possible evidence for analysis. Teachers become laboratory managers who approve/disapprove whether a forensic analyst group is allowed to proceed with their tests. The groups must show laboratory managers a write-up of laboratory procedures in order to justify the need to run the test. Once all evidence has been analyzed, the groups become expert witnesses in a court room setting. In court, teachers serve as judges and lawyers. Students must present their evidence and act as experts in their field. Through this method of assessment, students constantly reflect on their procedures, their results, and are held accountable for their experiments. As teachers, not judges, this type of assessment is informative because students understanding/lack of understanding will show depending on their ability to explain the evidence and its analysis. Because students have to solve the crime, they will have to employ all the skills learned to be able to explain their justification as to whether the "suspect" is guilty or not guilty. Thus, students are asked to mimic the real-world applications of their learning and make connections with the science content they have learned.

Bibliography

Bowen, R.. "Agarose Gel Electrophoresis of DNA." Biotechnology and Genetic Engineering. http://arbl.cvmbs.colostate.edu/hbooks/genetics/biotech/gels/agardna.html (accessed July 14, 2013). Section related to use of agarose use was helpful.

Butler, John M.. "DNA Biology Review." In *Forensic DNA typing biology, technology, and genetics of STR markers*. 2nd ed. Amsterdam: Elsevier Academic Press, 2005. 36-50. Great overview of DNA Biology with a broad range of topics and some depth.

Butler, John M.. *Forensic DNA typing biology, technology, and genetics of STR markers*. 2nd ed. Amsterdam: Elsevier Academic Press, 2005. Great overview of DNA Biology with a broad range of topics and some depth.

Butler, John M., Michael D. Coble, and Peter M. Vallone. "STRs Vs. SNPs: Thoughts On The Future Of Forensic DNA Testing." *Forensic Science, Medicine, and* Pathology 3, no. 3 (2007): 200-205. Good for background on SNPS.

"Chelex® 100 Extraction Process." National Forensic Science Technology Center . http://www.nfstc.org/pdi/Subject03/pdi_s03_m03_01.htm (accessed July 28, 2013). A guide to Chelex's use.

Clark, David P.. "The Polymerase Chain Reaction." In Molecular Biology Understanding the Genetic Revolution.. Burlington: Elsevier, 2005. 634-654. PCR in detail.

"DNA Evidence: Basics of Analyzing | National Institute of Justice." National Institute of Justice: Criminal Justice Research, Development and Evaluation. http://www.nij.gov/topics/forensics/evidence/dna/basics/analyzing.htm (accessed June 21, 2013). Background on the use of DNA as evidence.

"DNA Identification in Mass Fatality Incidents." President's DNA Initiative. http://massfatality.dna.gov/Chapter2/ (accessed May 13, 2013). Describe the beginning of collecting DNA information due to mass fatality incidents.

"DNA Technology - The science behind DNA testing." DNA Junction - Your resource for DNA testing information. http://www.dnajunction.com/technology/ (accessed June 27, 2013). Source for DNA testing uses, but not for background information.

"DNA Timeline: DNA Science from Mendel to Today." DNA Interactive: Discovering the DNA Structure and beyond. http://www.dnai.org/timeline/ (accessed June 22, 2013). Good site for student and teachers gives a wide scope and sequences DNA through time.

"DNA-RNA-Protein." Nobelprize.org. http://www.nobelprize.org/educational/medicine/dna/b/translation/translation.html (accessed June 23, 2013). Detailed overview of the Central Dogma.

"DNA.gov: Types of DNA Samples Suitable for Testing." The DNA Initiative. http://www.dna.gov/basics/analysis/types-of-samples (accessed June 21, 2013). National Institute of Justice site for DNA used in criminal cases.

Saltzman, Mark. "Genetic Engineering ." Seminar Discussion, Intensive Session from Yale National Initiative, New Haven, July 10, 2013.

Hayes, Molly, and Frieda Reichsman. "DNA Forensics." In *DNA and biotechnology*. 3rd ed. Burlington, MA: Academic Press/Elsevier, 2010. 173-190. Background on genetic principles used in biotech.

Houck, Max M., and Jay A. Siegel. "DNA Analysis." In *Fundamentals of forensic science*. Amsterdam: Elsevier/Academic Press, 2006.

265-293. Sections of the book are particularly helpful in giving chemistry, biology background as related to forensics.

Houck, Max M., and Jay A. Siegel. "Electrophoresis." In *Fundamentals of forensic science*. Amsterdam: Elsevier/Academic Press, 2006. 149-152. Sections of the book are particularly helpful in giving chemistry, biology background as related to forensics.

Roche. "How PCR Works." Roche Molecular Diagnostics - IVD Assays and Instruments. http://molecular.roche.com/About/pcr/Pages/PCRProcess.aspx (accessed July 13, 2013). PCR specifically as used at Roche.

Jobling, Mark A., and Peter Gill. "Encoded Evidence: DNA In Forensic Analysis." *Nature Reviews Genetics* 5, no. 10 (2004): 739-751. Short article giving basic overview of DNA forensics.

Kirby, Lorne T.. "DNA Amplification." In *DNA fingerprinting: an introduction*. New York: Stockton Press, 1990. 1-16. Good information but dated

Kirby, Lorne T.. "Genetic Principles." In *DNA fingerprinting an introduction*. New York: Oxford University Press, 1993. 7-33. Basic but dated information.

MacPherson, M. J., and S. G. MÃ¸ller. "Understanding PCR." In *PCR*. repr ed. Oxford: BIOS, 2003. 21-33. Overview of PCR and its purpose.

Morling, Niels. "PCR In Forensic Genetics." *Biochemical Society Transactions* 37, no. 2 (2009): 438. Article specifying use of DNA in forensics.

"Mount Pleasant High - Reports (CA Dept of Education)." DataQuest (CA Dept of Education). http://data1.cde.ca.gov/dataquest/DQReports.asp?CDSType=S&CDSCode=43694274334900 (accessed June 22, 2013).School data source.

Mount Pleasant. "Ed-Data Website." Ed-Data. http://www.ed-data.k12.ca.us/App_Resx/EdDataClassic/fsTwoPanel.aspx?#!bottom=/_layouts/EdDataClassic//profile.asp?Level=07&County=43&district=69427&school=4334900&reportnumber=16 (accessed June 22, 2013). School data resource.

Nicholl, Desmond S. T.. *An introduction to genetic engineering*. 1994. Reprint, Cambridge [England: Cambridge University Press, 2008.

Saltzman, W. Mark. "Biomolecular Principles: Nucleic Acids." In *Biomedical engineering bridging medicine and technology*. Cambridge: Cambridge University Press, 2009. 93-107. Text is a good overview of genetic principles/

"School Enrollment by Ethnicity - Enrollment by Ethnicity for 2012-13." DataQuest (CA Dept of Education). http://data1.cde.ca.gov/dataquest/Enrollment/EthnicEnr.aspx?cType=ALL&cGender=B&cYear=2012-13&Level=School&cSelect=MT%2E+PLEASANT+HIGH—EAST+SIDE+UNION—4369427-4334900&cChoice=SchEnrEth (accessed June 23, 2013). Information for MPHS and other high school data needed.

Sobrino, B, M Brion, and A Carracedo. "SNPs In Forensic Genetics: A Review On SNP Typing Methodologies." *Forensic Science International* 154, no. 2-3 (2005): 181-194. Discusses benefits and limitations of SNP typing.

"The Innocence Project - Home." The Innocence Project - Home. http://www.innocenceproject.org/ (accessed June 13, 2013). Resource for criminal cases overturned by new DNA information.

"What Is A Gene? | TechNyou." Welcome to TechNyou science education resources. | TechNyou.

http://education.technyou.edu.au/view/91/94/what-gene (accessed June 28, 2013). Website useful for a variety of information from genetic engineering, GMOs, and stem cells.

Teacher Resources Bibliography

"Common Core State Standards - Resources (CA Dept of Education)." California Department of Education. http://www.cde.ca.gov/re/cc/ (accessed July 16, 2013).

"DNA - The Double Helix - About." Nobelprize.org. http://www.nobelprize.org/educational/medicine/dna_double_helix/about.html (accessed July 15, 2013).

"DNA Replication." McGraw Hill. http://highered.mcgraw-hill.com/sites/9834092339/student_view0/chapter14/dna_replication.html (accessed July 16, 2013).

"Forensic Science | Bay Area Biotechnology Education Consortium." Bay Area Biotechnology Education Consortium. http://www.babec.org/node/71 (accessed July 16, 2013).

"Gel Electrophoresis Virtual Lab." Learn.Geneticsââ€ž¢. http://learn.genetics.utah.edu/content/labs/gel/ (accessed July 15, 2013).

"How Nucleotides Are Added in DNA Replication." McGraw Hill . http://highered.mcgraw-hill.com/sites/9834092339/student_view0/chapter14/how_nucleotides_are_added_in_dna_replication.html (accessed July 15, 2013).

"Structural Basis of DNA Replication." McGraw Hill. http://highered.mcgraw-hill.com/sites/9834092339/student_view0/chapter14/structural_basis_of_dna_replication.html (accessed July 16, 2013).

Chicago formatting by BibMe.org.

Appendix 1

CA State Common Core Standards

Writing Standards for Literacy in History/Social Studies, Science, and Technical Subjects

Grades 11 & 12

1. Write arguments focused on discipline-specific content.

b. Develop claim(s) and counterclaims fairly and thoroughly, supplying the most relevant data and evidence for each while pointing out the strengths and limitations of both claim(s) and counterclaims in a discipline-appropriate form that anticipates the audience's knowledge level, concerns, values, and possible biases.

e. Provide a concluding statement or section that follows from or supports the argument presented.

10. Write routinely over extended time frames (time for reflection and revision) and shorter time frames (a single sitting or a day or two) for a range of discipline-specific tasks, purposes, and audiences.

Reading Standards for Literacy in Science and Technical Subjects 6–12

3. Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks; analyze the specific results based on explanations in the text.

9. Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.

Appendix 2

Laboratory Research Notebook Guidelines by M. Okuda, R. Wou, V. Vitug

A laboratory notebook is essentially a factual account of work performed in the laboratory; it may also include the writer's interpretation of the results. Most laboratory notebooks are bound, and their pages are quadrille (marked with a square grid of lines), this is the preferred notebook used in this class. However for the purposes of this class, a college ruled composition book is also acceptable.

General Guidelines for your laboratory notebook:

1. ONLY write in black or blue ink-pen.

2. Laboratory Research Notebooks must stay in the Lab at all times.

3. The project managers (PM) must initial then highlight all COMPLETED labs in the lab book next to the conclusion section of the lab so that the initial and date is written across the interface so that a portion of the initials/date are on the taped-in portion as well as the original notebook paper.

4. When making entries use both sides of the research notebook paper.

5. Do not tear out pages from the laboratory notebook and do not write down notes from class lectures, labs, or CSI cases in the laboratory notebook.

6. No stapled papers can be taped in the lab notebooks and only one sheet of pre-written lab can be taped per page in the lab notebook

7. Erasures or liquid paper should not be made in the laboratory notebook. When corrections are in order, draw a single line through the material to be deleted and write the correction neatly above or beside the deleted material. Although this procedure may detract somewhat from the neatness of the report, the completeness and accuracy of the report should receive first priority. Therefore, do not hesitate to make corrections in this manner. The procedure is quite permissible.

8. Each section of the notebook should have a clear, descriptive heading, and sufficiently legible to be read and understood by any knowledgeable individual. Some or all of the following items should be included:

- The experiment *Title*
- A clear, concise statement of what the experiment *Purpose*
- A list of the supplies, equipment needed for the experiment under *Materials*
- Paste or write your *Procedures* for the lab activity

9. During the experiment, you should keep a detailed account of your work, reporting everything of importance that you actually did and saw. Your notes should not simply restate the textbook procedure but should describe in your own words how you carried out the experiment. You should include all relevant data such as the quantities of materials that you actually used (not the theoretical quantities that you calculated, unless they are exactly the same) and the results of any analyses you performed. Raw data should be recorded with particular care; if you forget to record data at the time you measure it, or if you recorded in incorrectly or illegibly, the results of an entire experiment may be invalidated.

10. List all persons from whom samples or data were obtained, shared, or transferred.

11. Title and number each page of your lab notebook according to the lab topic.

Appendix 3

Mock Case Summary by V. Vitug and R. Wou

Date: 2/12/13

Time: 10:45 PM

Victims: Alonso Acosta (Deceased)

Sophia Dixon

Suspect:Musa Awazie

Charges:1 st Degree Manslaughter

Breaking and Entering

Assault and Battery

Summary:

At approximately 10:45 PM, on 2/12/13, baseball starter Alonso Acosta arrived home with his wife, soap opera actress, Sophia Dixon saw signs of a break in. According to Sophia Dixon, Alonso Acosta pulls out a baseball bat to search for the intruder while she searches for her mobile phone to dial 911. Sophia Dixon states that her husband encounters the intruder and swung the baseball bat at the intruder, striking him. She was not

sure where the baseball bat struck the intruder due to it being very dark in the house.

Sophia Dixon went on record stating that she heard a single gun shot and something struck her on her head and that was the last thing she remembers. When she regained consciousness, she found her husband on the floor in the foyer with a pool of blood beneath him. She saw a handgun nearby and found her mobile to dial 911 for the paramedics and police. Alonso Acosta was pronounced deceased at the crime scene.

The suspect, Musa Awazie, was apprehended when he was identified by the security footage at the front gate of their estate on the night of the break in and homicide.

Biological Evidence:

1. Trace: Hair

2. DNA: Blood

3. Fingerprints

a. Baseball Bat

b. Crowbar

c. Front Door Knob

d. Gun

Physical Evidence:

1. Ballistics:

a. Bullet recovered inside drywall at end of foyer (taken from crime scene)

b. Reference bullet from revolver at crime scene (taken from crime lab)

c. Reference bullet from semi-auto gun recovered at suspect's home

2. Blood Spatter on Wall in Foyer

3. Blood Droplets in Hallway Towards Back Patio Door

4. Bullet Trajectory Analysis

Appendix 4

Web Assignment – DNA Structure Review, DNA Replication, and Intro to PCR (student worksheet)

Play the Double Helix

I. Game to refresh your memory regarding base pairing.

a. http://www.nobelprize.org/educational/medicine/dna_double_helix/about.html

II. Complementary Base Pairing of DNA

a. Click or Copy the link to the Mcgraw Hill video site.

http://highered.mcgraw-hill.com/sites/9834092339/student_view0/chapter14/structural_basis_of_dna_replication.html

b. Watch the video. Read along with the captions.

c. Take the quiz.

d. Send Ms. Vitug your results. Be sure to send your results to get credit for this part of the assignment.

III. DNA Replication

a. Click or Copy the link to the Mcgraw Hill video site.

http://highered.mcgraw-hill.com/sites/9834092339/student_view0/chapter14/dna_replication.html

b. Watch the video. Read along with the captions.

c. Take the quiz.

d. Send Ms. Vitug your results. Be sure to send your results to get credit for this part of the assignment.

IV. Addition of Nucleotides to template strands

a. Click or Copy the link to the Mcgraw Hill video site.

http://highered.mcgraw-hill.com/sites/9834092339/student_view0/chapter14/how_nucleotides_are_added_in_dna_replication.html

b. Watch the video. Read along with the captions.

c. Take the quiz.

d. Send Ms. Vitug your results. Be sure to send your results to get credit for this part of the assignment.

V. PCR (Polymerase Chain Reaction)

a. Click or copy the link

http://learn.genetics.utah.edu/content/labs/pcr/

VI. Run through the lab and its procedures pay attention to the details of PCR since this will be the focus of the next few weeks in class.

Appendix 5

Lab Activity – Micropipette Activity, By M. Okuda, modified by V. Vitug

Purpose: Practice using a 2-20ul micropipette

Materials – 4 dye colors 100ul in each vial, 1 (2-20ul) micropipette, wells (gridded stock paper)

Procedures/Protocols:

1. Obtain materials

2. Each student completes a row; note row, column & dye color;

3. Pipette only 7 ul per "well"

4. Read the hidden message and record

row	column	color
A	1	GREEN
A	2	GREEN
A	3	GREEN
A	5	GREEN
A	6	GREEN
A	7	GREEN
A	9	GREEN
A	10	GREEN
A	11	GREEN
B	1	BLUE
B	4	BLUE
B	5	BLUE
B	9	BLUE
B	12	BLUE
C	1	RED
C	2	RED
C	3	RED
C	5	RED
C	9	RED
C	10	RED
C	11	RED
D	1	GREEN
D	5	GREEN
D	9	GREEN
D	11	GREEN
E	1	BLUE
E	5	BLUE
E	6	BLUE
E	7	BLUE
E	9	BLUE
E	12	BLUE

Message Reads:

6 card message reads: _____

Conclusion: (Answer in a paragraph below)

Were you able to read the message? Where all the spots even in size and general shape, if not why? Describe the correct technique for pipetting.

Notes

1. Encoded Evidence, 740

2. Innocence Project Statistics

3. CA Department of Education, School Enrollment

4. Ibid, English Learners

5. Ed-Data, AYP and API

6. Kirby – Genetic Principles

7. Nicholl, 18

8. Saltzman YNI Seminar, July 10, 2013

9. TechNYou

10. Nicholl, 15

11. Nobel Prize.org, transcription

12. Ibid, translation

13. DNA and Biotechnology, 173

14. Ibid, 175

15. Nicholl, 52

16. Houck, 271

17. Ibid, 272

18. Butler, Forensic DNA Typing, 53

19. STRs vs. SNPs: thoughts on the future of forensic DNA testing, 201

20. Ibid, 202

21. Ibid, 178

22. Ibid, 180

23. Ibid, 279

24. Butler, Forensic DNA Typing, 41

25. Ibid, 30

26. NFSTC DNA analyst training, Chelex extraction

27. Ibid, 31

28. Ibid, 280

28. *Ibid*, 138

29. Ibid, 42

30. Ibid, 138

31. Ibid, Polyacrylamide gel, 138

32. Ibid, 143

33. M. Okuda, Forensic Science Supplementary Activities

34. Learn Genetics, Gel Electrophoresis