This packet presumes you already know the basics of DNA structure and function, from your Biology or Life Science class (a prerequisite for this course). If you need a refresher, there are innumerable websites which can help you, as well as books in the library. I would also be happy to check you out a Biology textbook over the break.
You are required to know the following for our exam on DNA in Forensics:

1. What is DNA and why is it important to forensic scientists?
2. What are genes and what is their function? What are genes made of, and where are they located?
3. DNA is a large molecule created by linking a series of repeating units. What is this type of molecule called? What are the repeating units?
4. Describe the basic structure of DNA. What is the name given to this type of structure?
5. Name the four bases associated with DNA. How are the bases paired on the DNA molecule?
6. How are proteins made? What determines the shape and function of a protein molecule?
7. What is the human genome?
8. Briefly describe the process of DNA replication.
9. What is PCR? Why is it useful to forensic scientists?
10. What are short tandem repeats? How are they useful to forensic scientists?
11. The forensic science community has standardized on 13 STRs for analysis. Give a few examples, and indicate what *your* STRs might be.
12. Approximately how many DNA-bearing cells are needed to obtain an STR profile?

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Polymerase Chain Reaction (PCR)

For nearly a decade, RFLP was the dominant DNA-typing procedure in the United States. However, its utility quickly ended by the mid-1990s. What caused this change? The answer is quite simple: the emergence of a revolutionary and elegant technique known as polymerase chain reaction, or PCR. Put simply, PCR is a technique designed to copy or multiply DNA strands. For the forensic scientist, who is often presented with minute quantities of materials, the opportunity to multiply the quantity of sample available for analysis was too good to pass up.

The PCR Process

PCR is the outgrowth of knowledge gained from an understanding of how DNA strands naturally replicate within a cell. The most important feature of PCR is the knowledge that an enzyme called *DNA polymerase* can be directed to synthesize a specific region of DNA. In a relatively straightforward manner, PCR can be used to repeatedly duplicate or amplify a strand of DNA millions of times. As an example, let's consider a segment of DNA that we want to duplicate by PCR:

–G–T–C–T–C–A–G–C–T–T–**C–C–A–G**–
–**C–A–G–A–G**–T–C–G–A–A–G–G–T–C–

To perform PCR on this DNA segment, short sequences of DNA on each side of the region of interest must be identified. In the example shown here, the short sequences are designated by boldface letters in the DNA segment. These short DNA segments must be available in a pure form known as a primer if the PCR technique is going to work.

primer
A short strand of DNA used to target a region of DNA for replication by PCR.

The first step in PCR is to heat the DNA strands to about 94°C. At this temperature, the double-stranded DNA molecules separate completely:

–G–T–C–T–C–A–G–C–T–T–C–C–A–G–

–C–A–G–A–G–T–C–G–A–A–G–G–T–C–

WebExtra 9.3
Polymerase Chain Reaction
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The second step is to add the primers to the separated strands and allow the primers to combine, or hybridize, with the strands by lowering the test-tube temperature to about 60°C.

–G–T–C–T–C–A–G–C–T–T–C–C–A–G–
C–A–G–A

C–C–A–G
–C–A–G–A–G–T–C–G–A–A–G–G–T–C–

The third step is to add the DNA polymerase and a mixture of free nucleotides (*A, C, G, T*) to the separated strands. When the test tube is heated to 72°C, the polymerase enzyme directs the rebuilding of a double-stranded DNA molecule, extending the primers by adding the appropriate bases, one at a time, resulting in the production of two complete pairs of double-stranded DNA segments:

–G–T–C–T–C–A–G–C–T–T–C–C–A–G–
C–A–G–A–G–T–C–G–A–A–G–G–T–C–

–G–T–C–T–C–A–G–C–T–T–C–C–A–G
–C–A–G–A–G–T–C–G–A–A–G–G–T–C–

This completes the first cycle of the PCR technique, which results in a doubling of the number of DNA strands from one to two. The cycle of heating, cooling, and strand rebuilding is then repeated, resulting in a further doubling of the DNA strands. On completion of the second cycle, four double-stranded DNA molecules have been created from the original double-stranded DNA sample. Typically, twenty-eight to thirty-two cycles are carried out to yield more than one billion copies of the original DNA molecule. Each cycle takes less than two minutes.

Advantages of PCR

Why couldn't the PCR technology be applied to RFLP DNA typing? Simply put, the RFLP strands are too long, often containing thousands of bases. PCR is best used with DNA strands that are no longer than a couple of hundred bases. The obvious solution to this problem is to characterize DNA strands that are much shorter than RFLPs.

Another advantage in moving to shorter DNA strands is that they would be expected to be more stable and less subject to degradation brought about by adverse environmental conditions. The long RFLP strands tend to break apart under adverse conditions not uncommon at crime scenes.

From the forensic scientist's viewpoint, PCR offers a third distinct advantage in that it can amplify minute quantities of DNA, thus overcoming the limited-sample-size problem often associated with crime-scene evidence. With PCR, less than one-billionth of a gram of DNA is required for analysis. Consequently, PCR can characterize DNA extracted from small quantities of blood, semen, and saliva. The extraordinary sensitivity of PCR

allows forensic analysts to characterize small quantities of DNA that could never be detected by RFLP. For instance, PCR has been applied to the identification of saliva residues found on envelopes, stamps, soda cans, and cigarette butts.

Key Points

- Polymerase chain reaction (PCR) can amplify minute quantities of DNA. The technique evolved from an understanding of how DNA strands naturally replicate within a cell.
- PCR technology cannot be applied to RFLP DNA typing because RFLP strands are too long, often numbering in the thousands of bases. PCR is best used with DNA strands that are no longer than a couple of hundred bases.
- Long RFLP strands tend to break apart under the adverse conditions at many crime scenes. The shorter DNA strands used in PCR are more stable and less subject to degradation caused by adverse environmental conditions.

Short Tandem Repeats (STRs)

short tandem repeat (STR)
A region of a DNA molecule that contains short segments of three to seven repeating base pairs.

The latest method of DNA typing, short tandem repeat (STR) analysis, has emerged as the most successful and widely used DNA-profiling procedure. STRs are locations (loci) on the chromosome that contain short sequence elements that repeat themselves within the DNA molecule. They serve as helpful markers for identification because they are found in great abundance throughout the human genome.

STRs normally consist of repeating sequences of three to seven bases; the entire strand of an STR is also very short, less than 450 bases long. These strands are significantly shorter than those encountered in the RFLP procedure. This means that STRs are much less susceptible to degradation and are often recovered from bodies or stains that have been subject to extreme decomposition. Also, because of their shortness, STRs are an ideal candidate for multiplication by PCR, thus overcoming the limited-sample-size problem often associated with crime-scene evidence. Only one-billionth of a gram or less of DNA is required—1/50 to 1/100 the amount normally required for RFLP analysis.

To understand the utility of STRs in forensic science, let's look at one commonly used STR known as TH01. This DNA segment contains the repeating sequence *A–A–T–G*. Seven TH01 variants have been identified in the human genome. These variants contain five to eleven repeats of *A–A–T–G*. Figure 9–11 illustrates two such TH01 variants, one containing six repeats and the other containing eight repeats of *A–A–T–G*.

During a forensic examination, TH01 is extracted from biological materials and amplified by PCR as described earlier. The ability to copy an STR means that extremely small amounts of the molecule can be detected and analyzed. Once the STRs have been copied or amplified, they are separated by electrophoresis. By examining the distance the STR has migrated on the electrophoretic plate, one can determine the number of *A–A–T–G* repeats in the STR. Every person has two STR types for TH01, one inherited from each parent. Thus, for example, one may find in a semen stain TH01 with six repeats and eight repeats. This combination of TH01 is found in approximately 3.5 percent of the population.

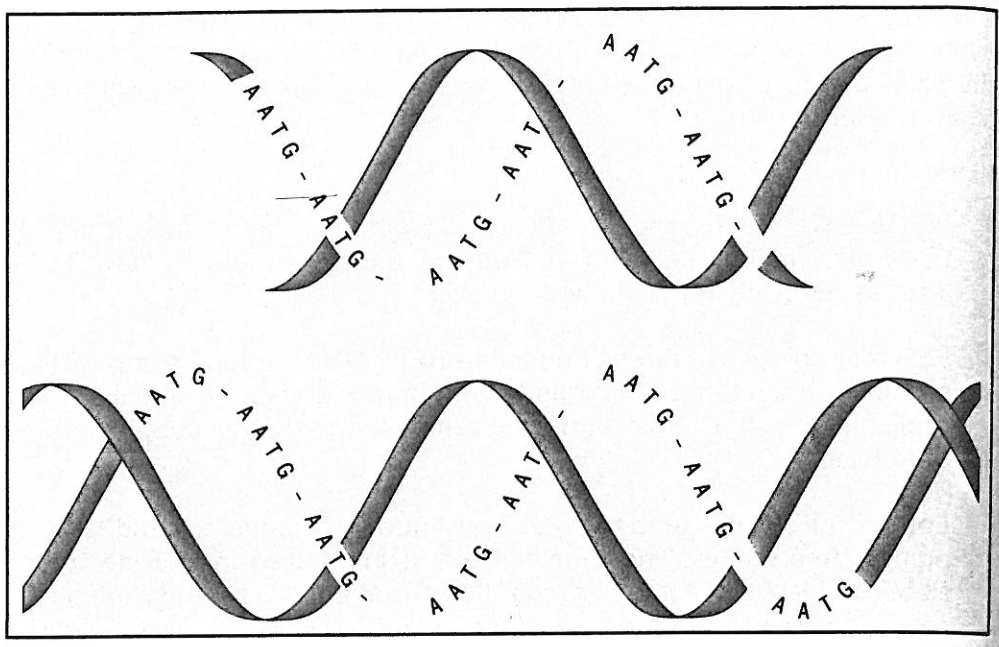

FIGURE 9–11 Variants of the short tandem repeat TH01. The upper DNA strand contains six repeats of the sequence *A–A–T–G;* the lower DNA strand contains eight repeats of the sequence *A–A–T–G.* This DNA type is known as TH01 6, 8.

Multiplexing

multiplexing
A technique that simultaneously detects more than one DNA marker in a single analysis.

What makes STRs so attractive to forensic scientists is that hundreds of different types of STRs are found in human genes. The more STRs one can characterize, the smaller the percentage of the population from which these STRs can emanate. This gives rise to the concept of multiplexing. Using PCR technology, one can simultaneously extract and amplify a combination of different STRs.

One STR system on the commercial market is the STR Blue Kit.[2] This kit provides the necessary materials for amplifying and detecting three STRs (a process called *triplexing*)—D3S1358, vWA, and FGA. The design of the system ensures that the size of the STRs does not overlap, thereby allowing each marker to be viewed clearly on an electrophoretic gel, as shown in Figure 9–12. In the United States, the forensic science community has standardized on thirteen STRs for entry into a national database known as the Combined DNA Index System (CODIS).

WebExtra 9.4
See the 13 CODIS STRs and Their Chromosomal Positions
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When an STR is selected for analysis, not only must the identity and number of core repeats be defined, but the sequence of bases flanking the repeats must also be known. This knowledge allows commercial manufacturers of STR-typing kits to prepare the correct primers to delineate the STR segment to be amplified by PCR. Figure 9–13 illustrates how appropriate primers are used to define the region of DNA to be amplified. Also, a mix of different primers aimed at different STRs will be used to simultaneously amplify a multitude of STRs (multiplexing). In fact, one STR kit on the commercial market can simultaneously make copies of fifteen different STRs.

DNA Typing with STRs

The thirteen CODIS STRs are listed in Table 9–1 along with their probabilities of identity. The probability of identity is a measure of the likelihood that two individuals selected at random will have an identical STR type.

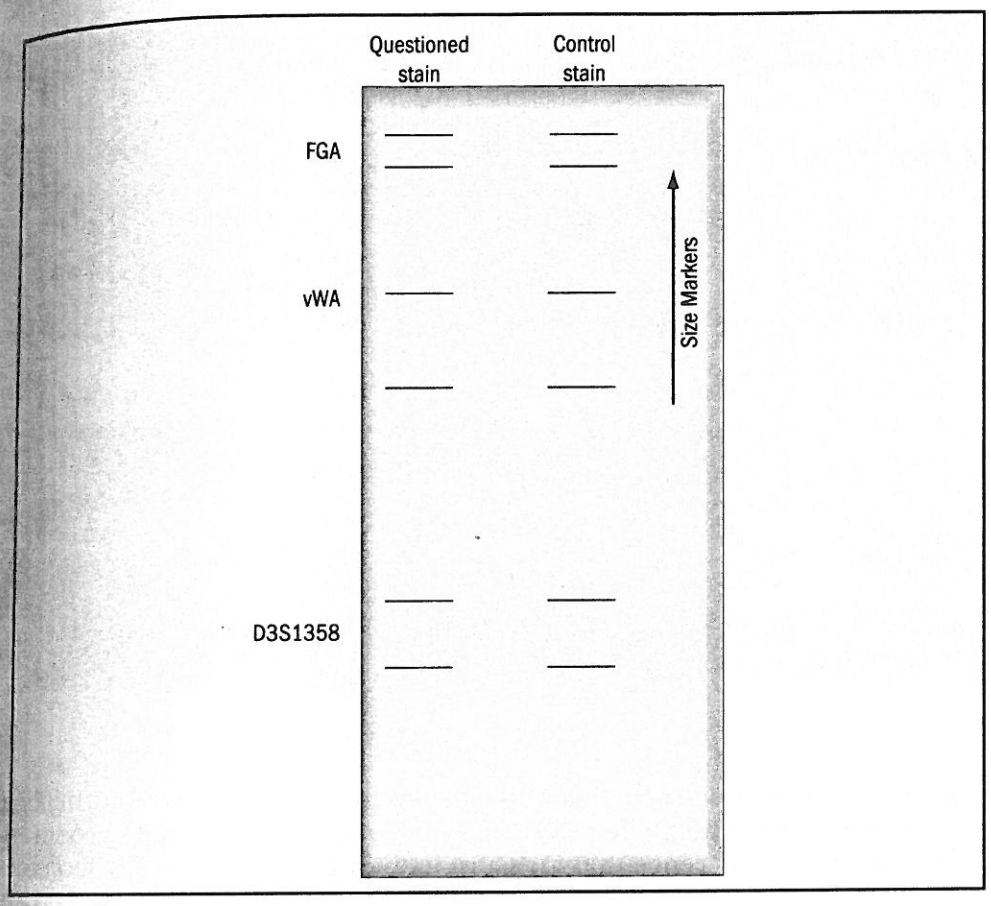

FIGURE 9–12 Triplex system containing three loci: FGA, vWA, and D3S1358, indicating a match between the questioned and the standard/reference stains.

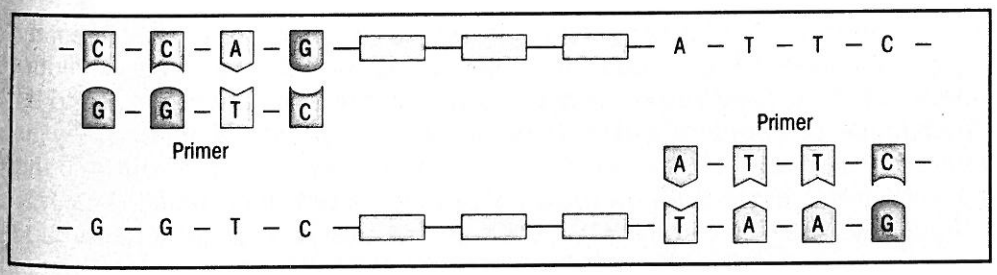

FIGURE 9–13 Appropriate primers flanking the repeat units of a DNA segment must be selected and put in place to initiate the PCR process.

The smaller the value of this probability, the more discriminating the STR. A high degree of discrimination and even individualization can be attained by analyzing a combination of STRs (multiplexing). Because STRs occur independently of each other, the probability of biological evidence having a particular combination of STR types is determined by the product of their frequency of occurrence in a population. Hence, the greater the number of STRs characterized, the smaller the frequency of occurrence of the analyzed sample in the general population.

The combination of the first three STRs shown in Table 9–1 typically produces a frequency of occurrence of about 1 in 5,000. A combination of the first six STRs typically yields a frequency of occurrence in the range of 1 in two million for the Caucasian population, and if the top nine STRs are

Table 9–1 The Thirteen CODIS STRs and Their Probability of Identities

STR	African-American	U.S. Caucasian
D3S1358	0.094	0.075
vWA	0.063	0.062
FGA	0.033	0.036
TH01	0.109	0.081
TPOX	0.090	0.195
CSF1PO	0.081	0.112
D5S818	0.112	0.158
D13S317	0.136	0.085
D7S820	0.080	0.065
D8S1179	0.082	0.067
D21S11	0.034	0.039
D18S51	0.029	0.028
D16S539	0.070	0.089

Source: *The Future of Forensic DNA Testing: Predictions of the Research and Development Working Group.* Washington, D.C.: National Institute of Justice, Department of Justice, 2000, p. 41.

determined in combination, this frequency declines to about 1 in one billion. The combination of all thirteen STRs shown in Table 9–1 typically produces frequencies of occurrence that measure in the range of 1 in 575 trillion for Caucasian Americans and 1 in 900 trillion for African-Americans. Importantly, several commercially available kits allow forensic scientists to profile STRs in the kinds of combinations cited here.

WebExtra 9.5
Calculate the Frequency of Occurrence of a DNA Profile
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Capillary Electrophoresis

The separation of STRs can typically be carried out on a flat gel-coated electrophoretic plate, as described earlier. However, the need to reduce analysis time and to automate sampling and data collection has led to the emergence of *capillary electrophoresis* as the preferred technology for characterization of STRs. Capillary electrophoresis is carried out in a thin glass column rather than on the surface of a coated-glass plate.

WebExtra 9.6
Understand the Operational Principles of Capillary Electrophoresis
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As illustrated in Figure 9–14, each end of the column is immersed in a reservoir of buffer liquid that also holds electrodes (coated with platinum) to supply high-voltage energy. The column is coated with a gel polymer, and the DNA-containing sample solution is injected into one end of the column with a syringe. The STR fragments then move through the column under the influence of an electrical potential at a speed that is related to the length of the STR fragments. The other end of the column is connected to a detector that tracks the separated STRs as they emerge from the column. As the DNA peaks pass through the detector, they are recorded on a display known as an *electropherogram.*

WebExtra 9.7
See the Electopherogram Record from One Individual's DNA
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Sex Identification Using STRs

amelogenin gene
A genetic locus useful for determining gender.

Manufacturers of commercial STR kits typically used by crime laboratories provide one additional piece of useful information along with STR types: the sex of the DNA contributor. The focus of attention here is the amelogenin gene located on both the X and Y chromosomes. This gene,