CHEM1200 Discovery in Biology

Practical

Polymerase Chain Reaction (PCR) and DNA Fingerprinting in Forensic Science

INTRODUCTION

DNA fingerprinting is a genetic technique that is used to determine the genotype (genetic makeup) of an individual. This technique allows us to analyze various DNA samples to determine whether they are from the same individual. In order to prove that two samples of DNA come from the same person, scientists compare genetic fingerprints (signature sequences in the genome) that differ from person to person. Some useful applications of DNA fingerprinting (also known as DNA profiling) include scientific investigations of crime scenes, missing persons, paternity testing and other legal proceedings.

Based on recent advances in DNA sequencing technologies, it is now known that the human genome consists of 3×10^9 nucleotides and it is estimated that there is ~1% difference in DNA sequence between individuals. These variable DNA regions in the human genome are known as DNA polymorphisms (i.e. many forms), which can be used by forensic scientists to generate unique DNA fingerprints for different individuals. One type of DNA polymorphism that is commonly used in forensic science is the Short Tandem Repeat (STR) polymorphism, which consists of a short stretch of DNA sequence (e.g. AATG, **Figure 1**) that is repeated many times in a row. STR is a type of length polymorphism (i.e. tandem DNA repeats of different lengths), and each of the STR site differs widely in length (which typically contain from 3 to 50 four-nucleotide repeats in tandem) between individuals. There are many of such polymorphic chromosomal regions in the human genome which differ in length between different individuals. For example, on human chromosome 11, a STR locus, TH01, consisting of a 4-bp DNA repeat sequence, $[AATG]_n$, has been reported. While one chromosome 11 allele is inherited from the mother, another copy is inherited from the father. It is now known that the TH01 locus is highly polymorphic and inherited in typical Mendelian fashion. So far, 21 different length polymorphisms in the TH01 locus have been observed in the human population. Figure 1 illustrates the DNA polymorphism of an individual who is carrying a (5, 9) genotype at the TH01 locus whereby one of the alleles contains 5 [AATG] repeats while the second allele harbors 9 [AATG] repeats.

…TTC CCT AATG AATG AATG AATG AATG CAC CAT… 5× [AATG] repeats

(Allele 1)

…TTC CCT AATG AATG AATG AATG AATG AATG AATG AATG AATG CAC CAT… 9× [AATG] repeats

(Allele 2)

Figure 1. STR polymorphism in the TH01 locus of a genotype (5, 9) person.

To perform STR genotyping in forensic investigations, DNA may be obtained from hair root follicle cells, cheek cells, blood, semen, saliva, bones, or skin. In most situations, only a small amount of DNA could be obtained at the crime scene for DNA fingerprinting. To overcome this limitation, the Polymerase Chain Reaction (PCR) technique has been routinely used in recent years. The PCR method was invented by Dr. Kary Mullis in 1985, and he was awarded the Nobel Prize in Chemistry in 1993 in recognition of his improvement of the PCR technique. The technique operates like a "DNA copier" where millions of copies of a DNA target are generated from just a single copy of DNA in a biological sample. Using this technique, the DNA region spanning a specific STR locus on a chromosome can be amplified ~10^6 fold in just one hour (**Figure 2**).

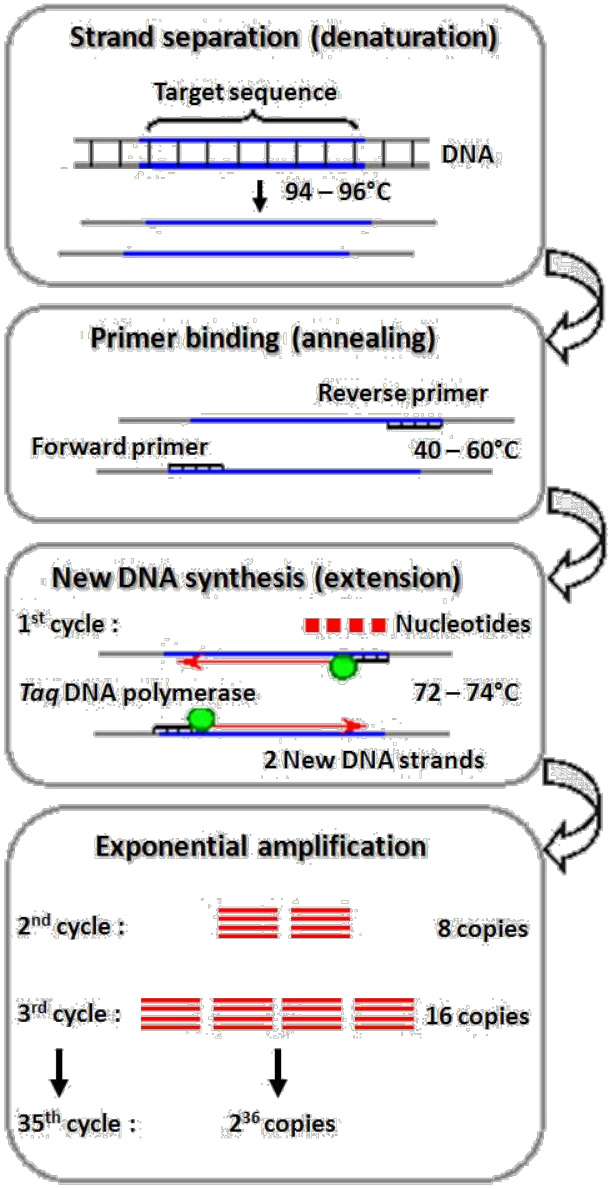

Figure 2. Principle of the polymerase chain reaction (PCR) technique. The events of PCR are outlined, with the DNA to be amplified and the "raw materials" needed for PCR to occur. (Adapted from the National Center for Biotechnology Information)

Many STR loci are scattered throughout the human genome, and hence can serve as potential targets for DNA fingerprinting. In current practice, DNA fingerprinting by STR analysis is performed on 13 predetermined STR sites to improve the power of discrimination in forensic investigations. In the human population, a huge variation exists within the 13 standard STR sites such that a DNA fingerprint based on these sites can definitively identify a single individual from within the entire human population. In the US, DNA profiles (fingerprints) from a variety of sources such as convicted criminals, biological samples from unsolved crime scenes, and missing persons are entered into the Combined DNA Index System

(CODIS) database (Figure 3) which is used by the Federal Bureau of Investigation (FBI) to help in their investigations and prosecutions of criminal cases.

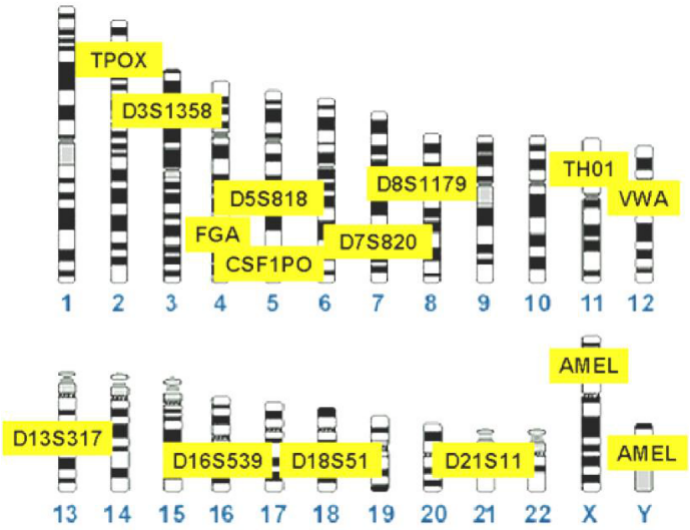

Figure 3. The 13 core CODIS STR loci and their genetic locations. (Adapted from the U.S. National Institute of Standards and Technology.)

In this practical, an exercise on forensic DNA fingerprinting will be carried out. DNA samples from blood stains at a crime scene and from four human suspects will be examined by PCR analysis of the TH01 STR locus (**Figure 1**). PCR primers targeting conserved DNA sequences flanking the TH01 STR locus will be used. The TH01 alleles in each DNA sample will be amplified by PCR to billions of copies and the PCR products of different sizes (based on the number of [AATG] repeats in the TH01 STR locus) analyzed by gel electrophoresis. Analysis of PCR products involves the following steps: (1) staining of DNA using a fluorescent dye – GelRed; (2) separating DNA of different sizes by agarose gel electrophoresis; and (3) detection of DNA bands on the UV transilluminator (Gel documentation system).

Learning Outcomes

Upon completion of this exercise, students should be able to:

1. Outline a procedure for carrying out the Polymerase Chain Reaction.
2. Describe the principles upon which PCR is based.
3. Analyze a human DNA fingerprint based on the TH01 locus.
4. List possible uses of DNA polymorphisms.

PART 1 – PCR amplification of the TH01 locus in human DNA

Materials Provided:

Class equipment/materials

PCR machines (2 x)

Microcentrifuges for 0.2-mL microtubes (4 x)

Group (per 4 students)

DNA samples (from Crime Scene and human suspects 1, 2, 3, and 4) – 15 µL of each Sterile water – 100 µL

Ice-cold PCR Master Mix + primers (MMP, blue tube) – 80 µL

Sterile 0.2-mL microtubes – 6 pieces

Microtube rack – 1 rack

Sterile pipette tips (10 µL) – 1 rack

Micropipettes (10 µL) – 1 set

Marker pen

Ice-bath

PROCEDURE

1. Label six microtubes (0.2 mL) – "CS", "S1", "S2", "S3", "S4", and "-ve" and include your group name as well.
2. Set up 20 µL PCR reactions by adding the components shown in **Table 1** to each of the labeled tubes.

   For example, to set up PCR reaction for the tube "CS", use a micropipette adjusted to 10 µL, transfer 10 µL of "DNA – Crime Scene" to the 0.2 mL tube labeled as "CS". Then, using a fresh tip, transfer 10 µL of "MMP" (blue tube) to the "CS" tube.

   Important: Use a fresh pipette tip for adding DNA sample and MMP solution. Cap each tube immediately after adding the MMP solution.

Table 1. PCR reaction mixtures prepared in this experiment.

Tube	DNA template	Master mix* + primers
CS	10 µL DNA sample – Crime Scene	10 µL MMP
S1	10 µL DNA sample – Suspect 1	10 µL MMP
S2	10 µL DNA sample – Suspect 2	10 µL MMP
S3	10 µL DNA sample – Suspect 3	10 µL MMP
S4	10 µL DNA sample – Suspect 4	10 µL MMP
-ve	10 µL sterile water	10 µL MMP

** Master mix (MMP) contains all reagents necessary for a PCR reaction to occur which includes dNTPs, Taq DNA polymerase and salts.*

3. Centrifuge the tubes at 10,000 rpm for 5 seconds (sec) to bring down all liquid to the bottom of the tubes and mix the solution.
4. Place all 6 tubes into a PCR machine.
5. Program the desired PCR profile into the machine as shown in **Table 2** below. The PCR program takes around 1.5 h to complete.

Table 2. PCR Cycling Profile.

Temperature	Time	Number of cycles
94 °C	2 min	1
94 °C	20 s	25
58 °C	20 s	
72 °C	1 min	
72 °C	10 min	1
End of program		

PART 2 – Gel electrophoresis and visualization of PCR products.

MATERIALS

Class equipment/materials

1% Agarose gel + GelRed solution

[**Note**: GelRed is a bio-safe fluorescent nucleic acid-binding dye that binds to nucleic acids (DNA and RNA). Under the UV light, GelRed gives out fluorescent light which indicates the presence of DNA.]

Allelic ladder (Marker)

Power pack supply

Horizontal gel electrophoresis assembly

GelDoc 1000 Imaging System (UV transilluminator)

Group (per 4 students)

Microtube rack – 1 rack

Sterile pipette tips (10 µL) – 1 rack

Micropipettes (10 µL) – 1 set

PROCEDURES

1. Load 10 µL of DNA sample from the PCR tubes **(from PART 1 experiment)** onto a 1% agarose gel in the following order:

Lane	DNA sample
1	Allellic ladder* (Done by demonstrator)
2	CS
3	S1
4	S2
5	S3
6	S4
7	-ve

** Allelic ladder (Marker) calibrates PCR product sizes to STR repeat number for genotyping purposes.*

2. Carry out gel electrophoresis of your DNA samples at 100 V for ~30 minutes.
   Important: During gel electrophoresis, take care that the orange dye (representing the smallest possible PCR product) does not migrate out of the gel.
3. Photograph the gel image of your DNA samples using the GelDoc 1000 Imaging System (UV transilluminator).
   (Steps 2 and 3 are performed by demonstrators)

Analysis of results

1. On the gel image that you obtained from your demonstrator, the number of STR repeats that are present in each band of the TH01 STR Allelic Ladder is labeled. For each PCR reaction, compare the bands in each lane to the Allelic Ladder. Record the genotype of each of your samples in table form.
2. Does the Crime Scene DNA sample have a genotype that matches any of the suspects? If so, which one?
3. Based on your results, which suspect(s) is/are not incriminated in this investigation? Explain your answer.