Module 7

Forensic Biology

Forensic Science Teacher Professional Development

Serology and Forensic DNA Module

MODULE 7A

Unit 1: Introduction & History of DNA Typing

Unit 2: DNA Biology Review

MODULE 7B

Unit 3: Sample Collection, DNA Extraction and DNA Quantitation

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Unit 4: The Polymerase Chain Reaction

Unit 5: Short Tandem Repeat Markers and Commercial Kits

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MODULE 7D

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Unit 8: Y-chromosome Analysis

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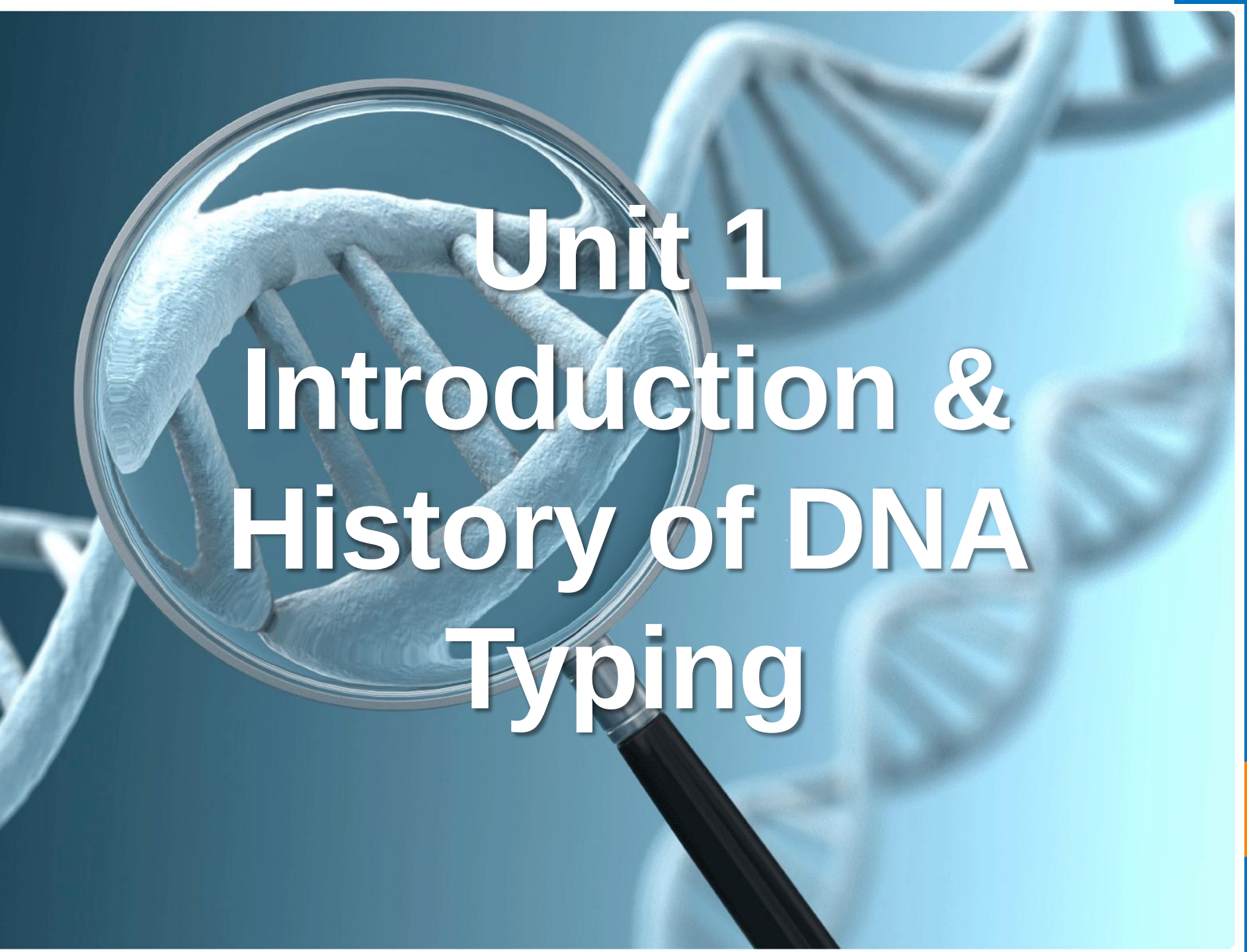
Module 7A

Introduction and the Structure of DNA

Forensic Science Teacher Professional Development


Unit 1
Introduction &
History of DNA
Typing

Part 1 A Case in Virginia

- On August 26, 1999, an assailant in Virginia robbed and raped a female student in front of her boyfriend.
- The assailant forced the female student to take a shower.
- After he accomplished his goal and feeling that nothing would incriminate him for this crime, he went to the kitchen and grabbed a beer.
- He drank the beer in peace and he left, unaware that he still had left an important piece of evidence that might link him to the crime scene.

Part 1 A Case in Virginia

- Saliva was collected from the beer can.
- Semen stains were also collected from the bed sheets.
- A DNA analysis was performed.
- A match was reported between the semen stains found in the bed and the beer can.
- Next, a suspect list from the sex offender registry of the area and 40 suspects' samples were sent to the DNA laboratory for analysis.
- Unfortunately, all of them were eliminated as possible contributors of the crime scene sample.
- At this point, the case was left open but no possible suspect was left as a link to this case.

Part 1 A Case in Virginia

- Two months later, a match was reported with a convicted offender sample from the Virginia database, submitted several years before.
- In April 2000, the intruder was found guilty of rape, forcible sodomy, and abduction. He was sentenced to a 90-year prison term. See the links below for more information on this case:

  http://www.cavalierdaily.com/1999/10/06/police-charge-suspected-armed-rapist/

  http://www.cavalierdaily.com/1999/10/08/suspects-fingerprints-match-rape-evidence/

This introductory case shows the importance and relevance of the DNA analysis in crime scene investigation. The usefulness of the DNA database is also evident to link DNA evidence to offenders and to associate different crime scenes.

Part 2 The First Use of Forensic DNA Testing

- In1983 and 1986, two young girls, Lynda Mann and Dawn Ashworth, were sexually assaulted and brutally murdered in Leicestershire, UK. You may click the link below to find out how this case is associated with forensic DNA.

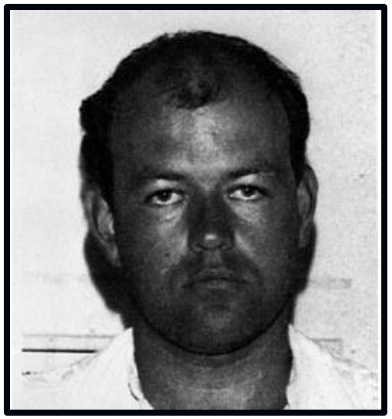
http://news.bbc.co.uk/2/hi/uk_news/england/leicestershire/7765210.stm

Part 2 The First Use of Forensic DNA Testing

- A local man confessed, but there was no DNA match with the samples collected at the crime scene.
- The police decided to perform a mass DNA screening in town.
- Four thousand samples from males living in the area were collected, but the DNA analysis resulted in no matches.
- This was the first time that a massive DNA collection was done in the history of forensic DNA analysis.

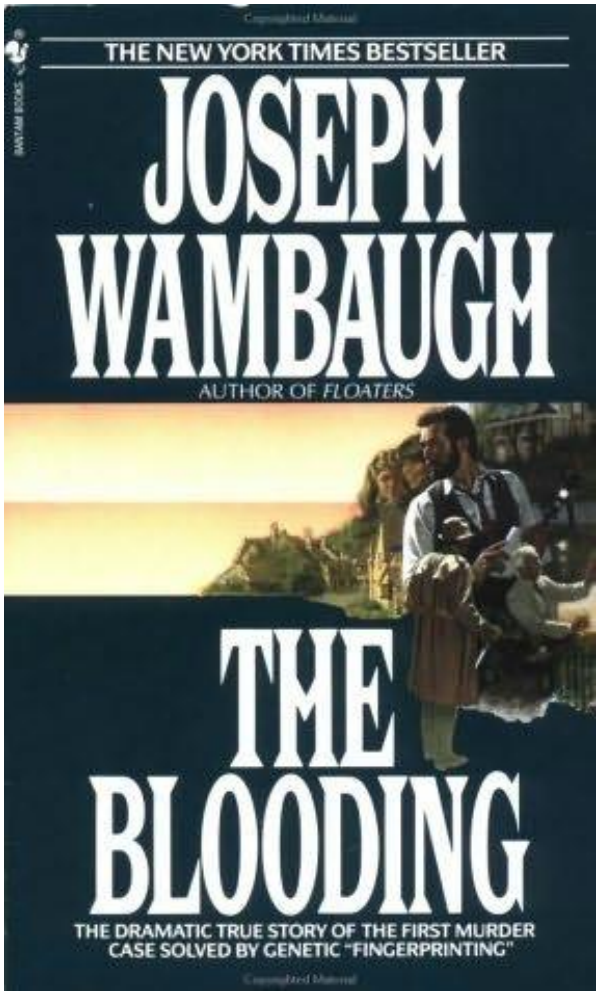
Part 2 The First Use of Forensic DNA Testing

- A witness in a bar heard that during the mass DNA sample collection, someone gave a sample for a friend.
- The name of the person who gave the sample was Colin Pitchfork. He was located and his blood was subjected to DNA analysis.
- Pitchfork's DNA matched the samples from both crime scenes.
- Colin Pitchfork was convicted and sentenced to life in prison.



Part 2 The First Use of Forensic DNA Testing

- The case became famous because it was the first time that DNA was used to convict a person.
- The Pitchfork case gained a lot of attention from the media, and the story was published in a book entitled *The Blooding*, by Joseph Wambaugh.



Part 3 History of DNA Typing

- Forensic DNA typing is the newest and the most powerful technique in forensic sciences and kinship analysis.
- In 1980 Wyman and White described the first polymorphic marker.
- This DNA marker is characterized by a number of restriction fragments of variable lengths called Restriction Fragments Length Polymorphisms, or RFLPs.

Part 3 History of DNA Typing

- “DNA Fingerprinting” was first described in 1985 in a paper entitled “Hypervariable Minisatellite Regions in Human DNA,” by Alec Jeffreys et al.
- Jeffreys and his team were analyzing the human myoglobin gene when they discovered that certain regions of DNA contained DNA sequences that were repeated next to each other.
- These repeated regions became known as VNTRs (Variable Number of Tandem Repeats).
- They also discovered that the number of repeats present in a sample were different between individuals.
- The technique used to examine the VNTRs was called RFLP.

Part 3 History of DNA Typing

- When this DNA is isolated, cut with restriction enzymes (like molecular scissors that cut the DNA in defined places), and hybridized with a probe consisting in a core repeat, a complex ladder of DNA fragments is obtained (see Figure 1).

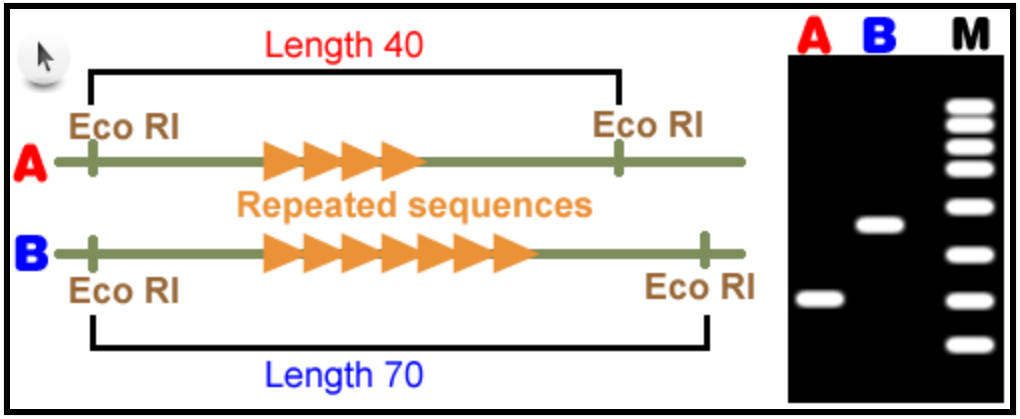


Figure 1
VNTRs scheme

Part 3 History of DNA Typing

- The RFLP methods were first used in an immigration case in the UK and then in the Pitchfork double homicide case (previously mentioned).
- Since then, human identification testing by means of DNA typing has been widespread.
- During the last twenty years, the use of DNA evidence in crime scene investigation and kinship analysis has grown significantly.
- Today, hundreds of public and private laboratories conduct hundreds of thousands of DNA tests annually in the United States. DNA testing programs are now run in five continents.

Part 3 History of DNA Typing

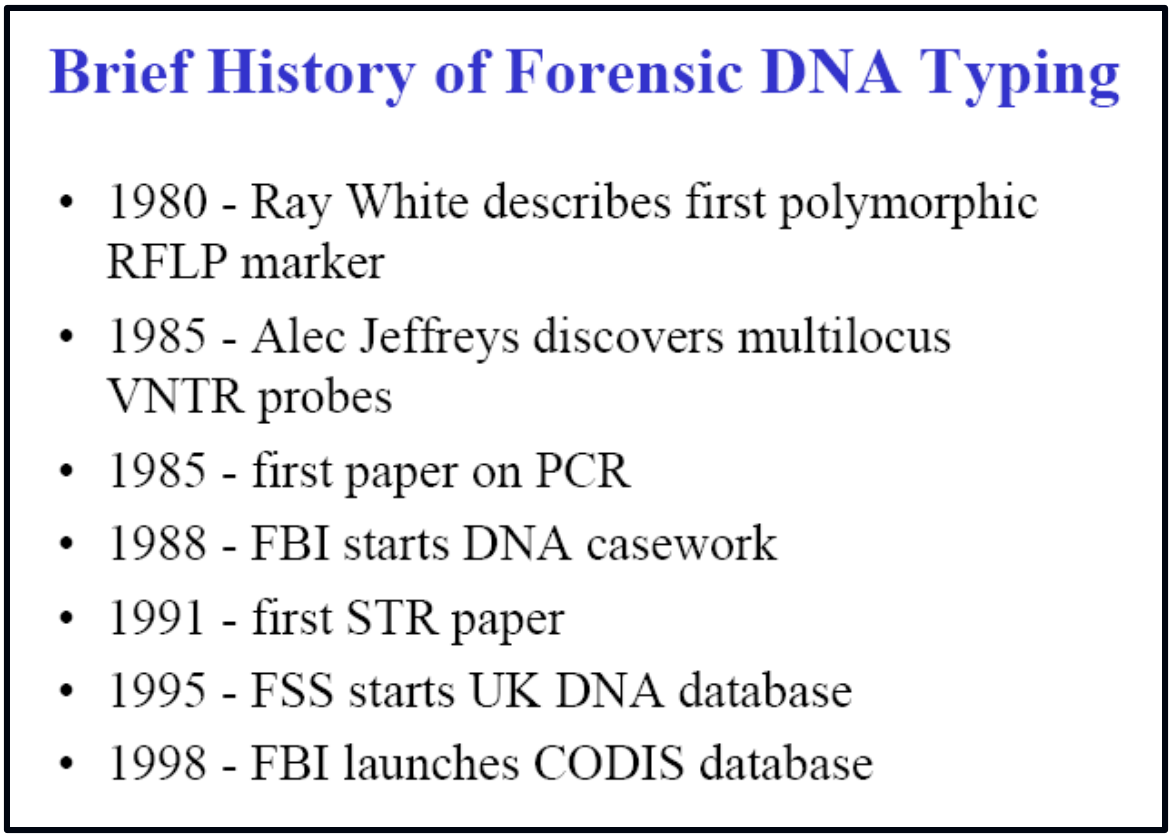


Figure 2

Brief history of DNA typing

http://www.cstl.nist.gov/strbase/ppt/intro.ppt

Part 3 History of DNA Typing

- Differences between technologies used to perform forensic DNA testing consist of the ability to discriminate between two individuals and the speed of analysis.
- Advances have occurred in the last 20 years.
  a) The speed of analysis improved from 6 to 8 weeks to perform an RFLP analysis to a few hours for a short tandem repeat (STR) analysis.
  b) There have been advances in terms of sample processing, speed, and sensitivity.
  c) For example, RFLP require a minimum of 25 nanograms (ng) of intact, undegraded DNA and STR requires only 1 ng of partially degraded DNA.
  d) Automation of the analysis also decreased the analysis time and in this way increased the throughput of sample tests.

Part 4 DNA Sample Processing Steps

- STRs are the most commonly used DNA markers today.
- They are a simplified version of the VNTRs described by Jeffreys et al.
- The sample obtained at the crime scene is subjected to different processes that include
  - biology,
  - technology, and
  - genetics.

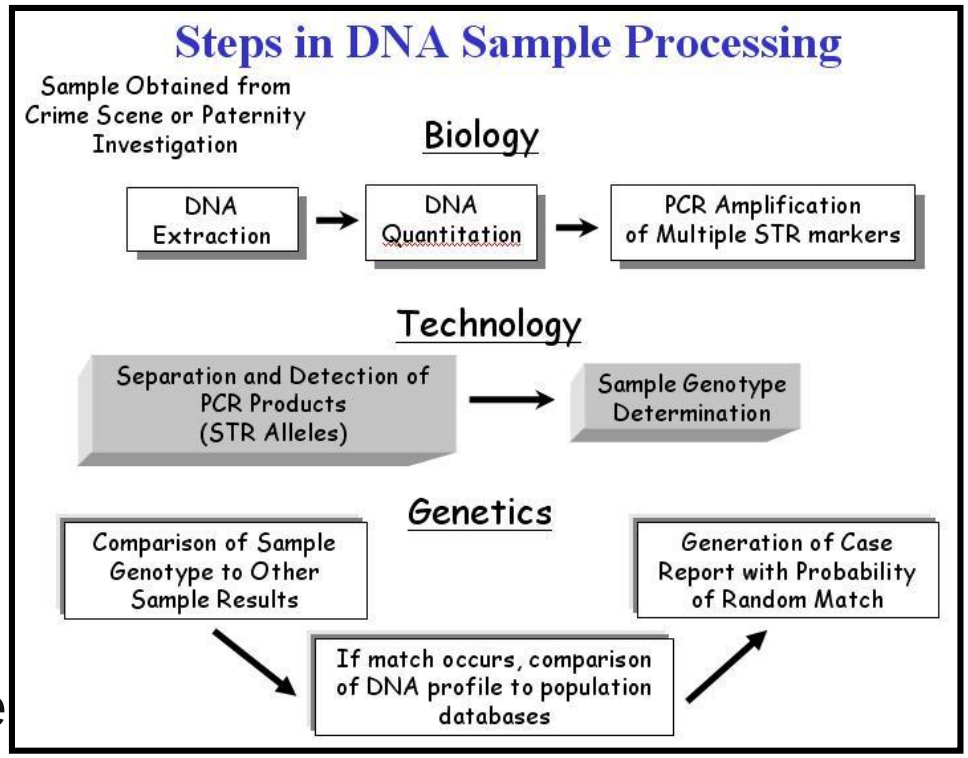


Figure 3
Steps in DNA sample processing

Part 4 DNA Sample Processing Steps

Biology

- The biological sample is extracted and the DNA is purified from its original matrix.
- The DNA is then quantified and specific regions are copied through an enzymatic reaction called polymerase chain reaction, or PCR.
- PCR produces millions of copies of the regions of interest, and in this way minute amounts of DNA can be analyzed simultaneously.

Part 4 DNA Sample Processing Steps

Technology

- After defined regions of the DNA are amplified (STRs), these fragments need to be separated by size.
- This process is accomplished by means of capillary electrophoresis (CE).
- Fluorescent methods increase the sensitivity of detection of the PCR products.
- The STR alleles are detected, and then the number of repeats in each amplified product is determined.
- The process is called sample genotyping. The most used instrument platform for fluorescent detection of STR products in the United States is the ABI Prism 310 Genetic Analyzer (capillary electrophoresis).

Part 4 DNA Sample Processing Steps

Genetics

- The resulting profiles or genotypes are compared between the evidence and the suspect or victim samples to verify if there is a match or not.
- DNA databases are important tools for law enforcement purposes.
- The STR profile obtained from the crime scene sample can also be searched against a DNA database of convicted felons or a database of DNA evidence from previous crime scenes.
- In the case of a paternity test, a child's genotype is compared to his or her mother's and the alleged father.

Part 4 DNA Sample Processing Steps

Genetics, continued

- If there is no match between the questioned sample and the reference sample, then the samples would be considered to be originated from different sources.
- This situation is called exclusion.
- If a match is verified between the questioned and the reference samples, then the DNA profile is compared to a population database.
- A population database is a collection of DNA profiles from unrelated individuals from different ethnic groups.
- It is very important to use the database corresponding to the ethnic group of the suspect or victim since genetic variation exists between different groups, such as African American, Caucasian, Hispanics, etc.

Part 4 DNA Sample Processing Steps

Genetics, continued

- When the DNA analysis is concluded and the match is verified, a case report or a paternity test result is generated.
- The report must include the random match probability (RMP) for the obtained match.
- The RMP is the chance that a randomly chosen individual from a population has the same STR profile with the markers analyzed.

Part 5
An Example of STR Multiplex Result

- In a single reaction, ten markers can be amplified simultaneously. Ten different sites, located in different regions of the genome, are targeted at the same time.
- The amplified products are separated by size using capillary electrophoresis and detected according to their fluorescent tags.
- The results can be shown as different peaks with different colors separated along the electropherogram (Figure 4).

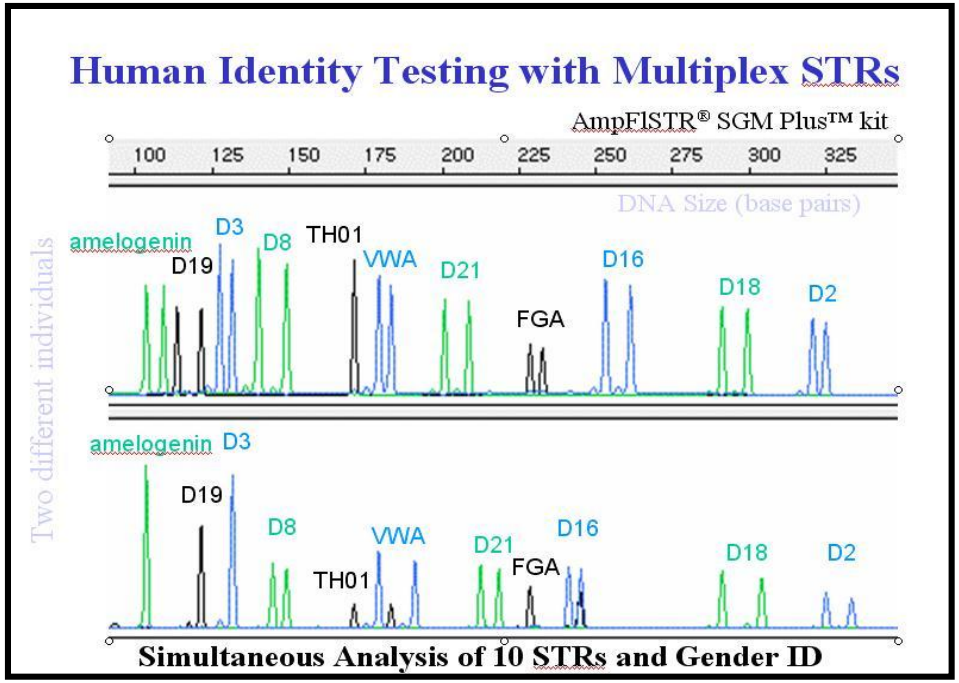


Figure 4
Electropherogram showing the different DNA profiles of two individuals using 10 STR markers

Part 5
An Example of STR Multiplex Result

- In the example depicted in Figure 4, two different individuals were genotyped.
- A male profile is shown in the upper panel and a female in the lower panel.
- Comparing the different profiles, the marker D19 shows two peaks for the male and one peak for the female.
- In the case of D2, both have two peaks, but they are located at different positions due to their different sizes.
- Using ten STR markers provides a random match probability of 1 in one trillion, and the sample size can be as little as a drop of blood.
- The amplified products, or peaks, were labeled during the amplification with fluorescent tags.

Part 5
An Example of STR Multiplex Result

- In this case, three colors were used: blue (markers D3, vWA, D16, and D2), green (amelogenin, D8, D21, and D18) and yellow, which is shown in black for visibility (D19, TH01, and FGA).
- It is important to note that the amelogenin was used for gender identification.
- The male has two peaks (XY); meanwhile the female has only one peak (XX).

Part 6 The Innocence Project

- In the same way that forensic DNA testing plays a role in implicating the guilty, its function is also to protect the innocent.
- During the last decades, DNA evidence was used to free wrongfully convicted people from prison.
- Due to these cases, some states like Illinois put capital punishment on hold after realizing that several inmates have been exonerated by post-conviction DNA testing.

Part 6 The Innocence Project

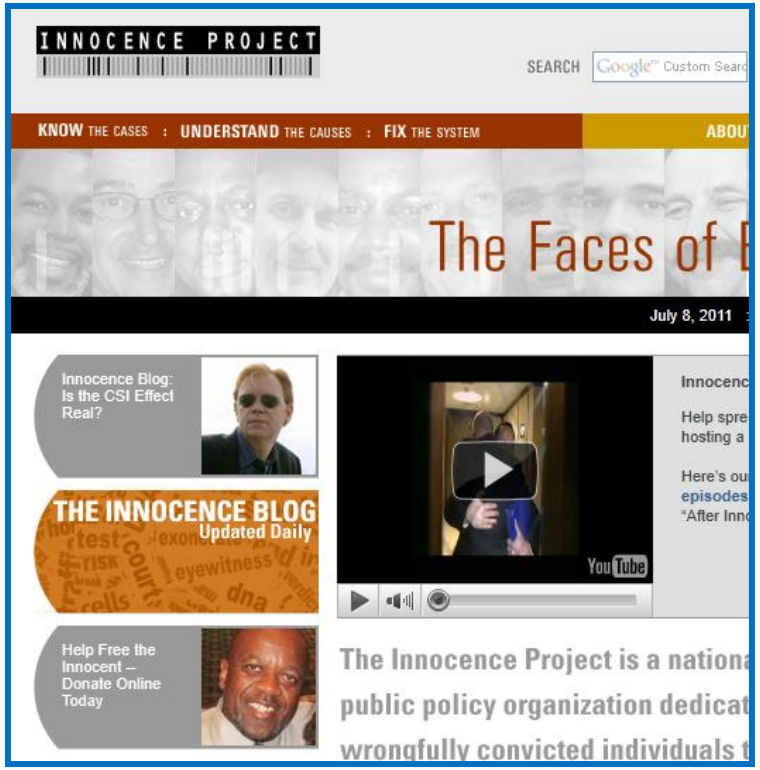
- As of May 2011, more than 175 people have been exonerated after being wrongfully convicted by misidentification.
- Fortunately, the evidence associated with these cases was preserved for many years and was used for post-conviction DNA testing.
- The post-conviction DNA testing results excluded these wrongfully convicted inmates as perpetrators of crimes.

Part 6 The Innocence Project

- In 1992, Barry Sheck and Peter Neufeld launched the Innocence Project at the Benjamin Cardozo School of Law in New York City.
- The Innocence Project is a non-profit organization dedicated to exonerating wrongfully convicted individuals through DNA testing and reforming the criminal justice system.
- This organization promotes cases where evidence is available for post-conviction DNA testing.
- The Innocence Project is also a network that includes more than 40 schools of law in the United States and Australia.

Part 6 The Innocence Project

- This group of lawyers screens thousands of cases requesting DNA testing to prove prisoners' innocence.
- Due to this initiative, legislation was promoted to fund post-conviction DNA testing at the state and federal level.
- You may read more about the Innocence Project at the website below:



http://www.innocenceproject.org/


Unit 2
DNA Biology
Review

Part 1 DNA Structure

- Cells are like micro factories; they
  - receive molecules as raw material,
  - synthesize new substances, and
  - discard the waste material.
- Every cell has the ability of
  - auto replication using the code of the deoxyribonucleic acid (DNA) as a template,
  - synthesizing new molecules, and
  - using the enzymes as catalysts.

Part 1 DNA Structure

- It was estimated that the human body is composed of 10^{14} cells containing a considerable amount of DNA.
- In eukaryotic cells, the majority of DNA is nuclear DNA (nDNA); only a small portion of extra-nuclear DNA is found in mitochondria and chloroplasts.
- The coded information contained in DNA is passed from generation to generation.
- DNA has two basic purposes:
  - To make copies of itself
  - To carry instructions about how to synthesize proteins

Part 1 DNA Structure

- DNA is composed of different units called nucleotides.
- Nucleotides have three parts:
  - Base
  - Sugar
  - Phosphate group
- The sequence of the bases are responsible for the variation in the human genome.

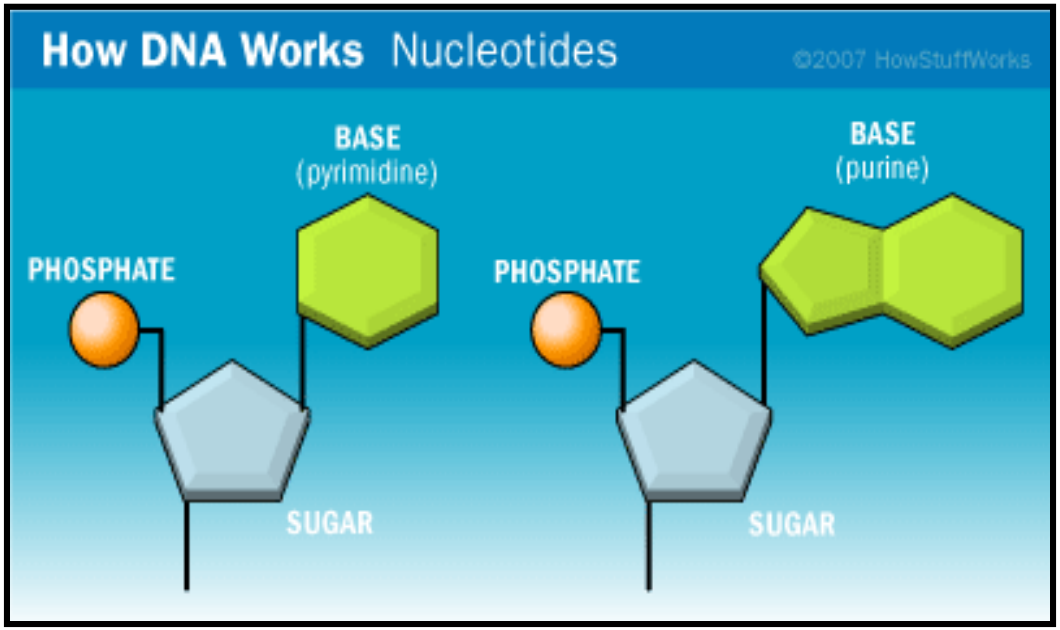


Figure 5
Structure of a nucleotide

Part 1 DNA Structure

- There are four types of bases:
  - Adenine (A)
  - Guanine (G)
  - Cytosine (C)
  - Thymine (T)
- There are three billion nucleotide positions in the human genome. With four possibilities for each position (A, T, C, or G), there are trillions of possible combinations.
- The coded information present in DNA depends on the order or sequence of its bases.

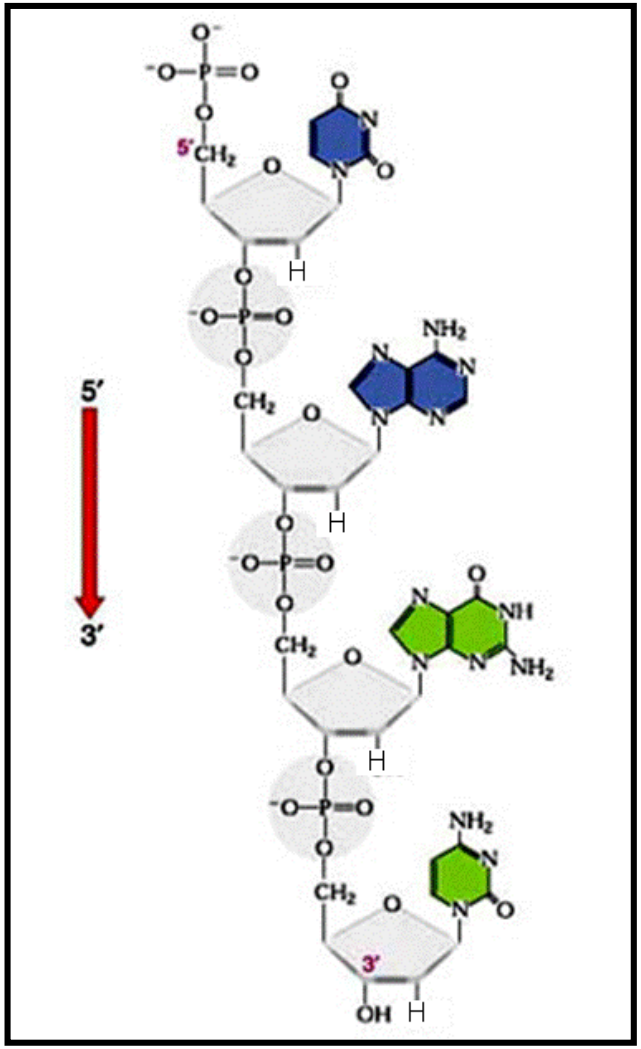
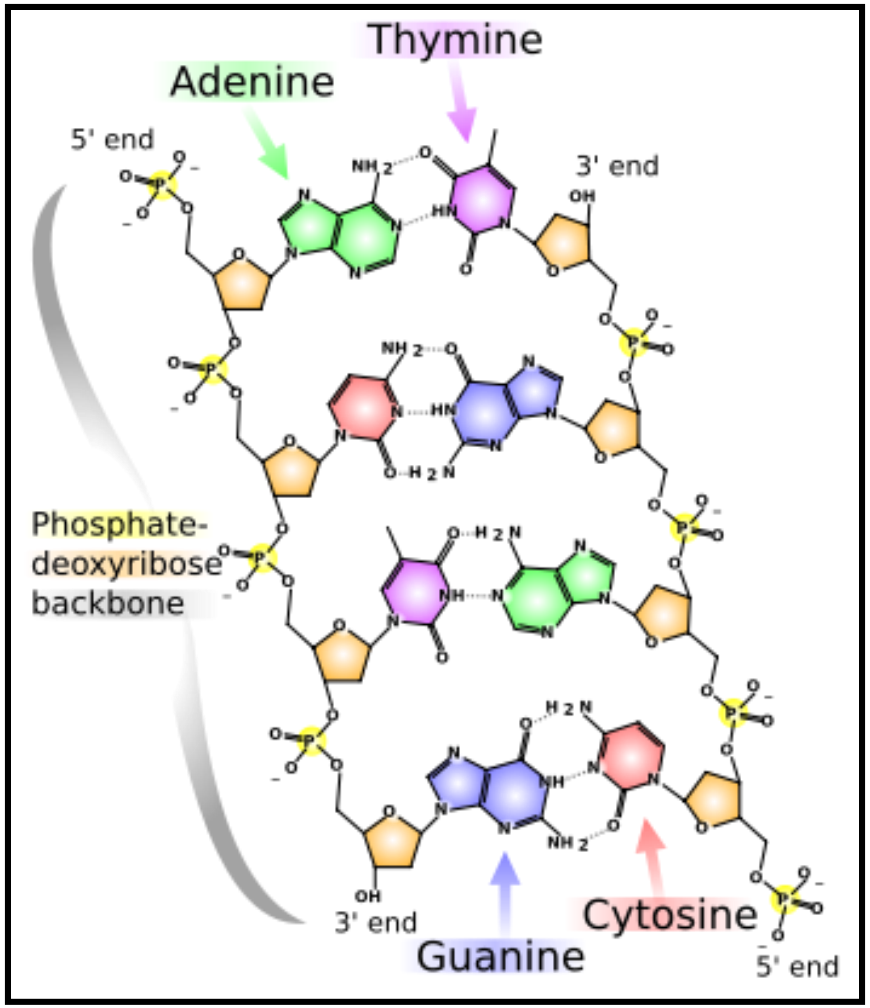


Figure 6
DNA sequence and 5’ – 3’ directionality

Part 1 DNA Structure

- The DNA is composed of two strands:
  - One parallel (5’ to 3’ direction)
  - One anti-parallel (3’ to 5’ direction relative to the parallel strand)
- The strands are linked together by a process called hybridization.
- According to the Watson and Crick model, complementary bases pair, or hybridize, together: adenine pairs with thymine, and guanine pairs with cytosine.
- The adenine-thymine pair contains two hydrogen bonds and the guanine-cytosine pair contains three hydrogen bonds.

Part 1 DNA Structure



- Due to the bonding of the nucleotides, the DNA forms a double helix structure.

Figure 7
Nucleotide pairing showing the number of hydrogen bonds in each pair

Part 1 DNA Structure

- The hydrogen bonds, responsible for the DNA double helix structure, can be broken by high temperatures (near 100°C) or by chemical treatment (like urea or formamide) in a process called denaturation.
- Denaturation is a reversible process.
- If the temperature is decreased, both strands will bind together again in a process know as reannealing.

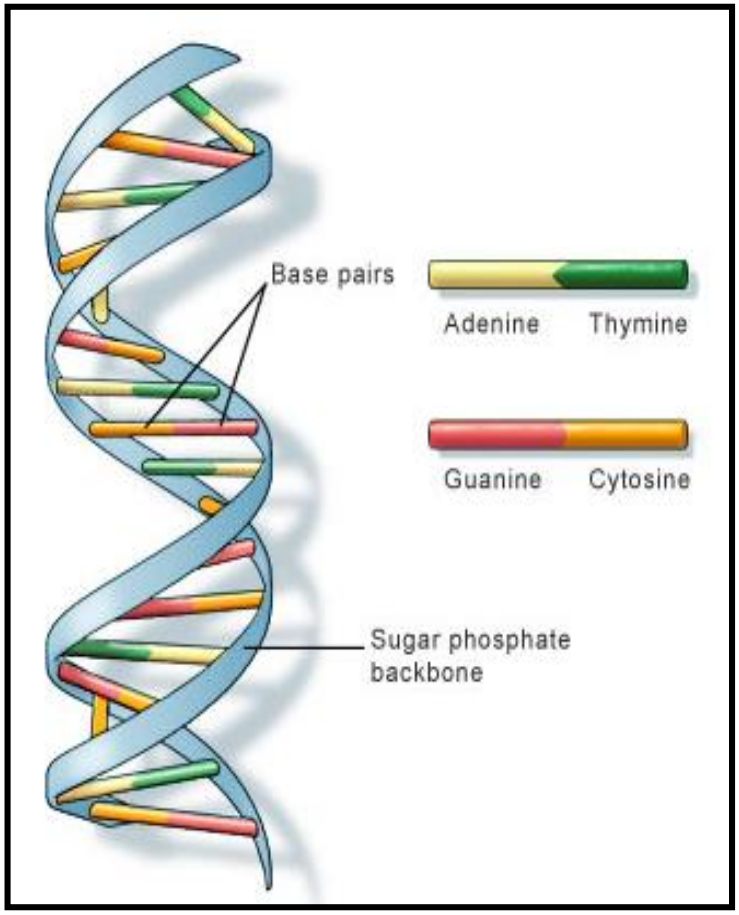


Figure 8
DNA double helix structure

Part 2 Organization of the DNA

- In eukaryotic cells, the nuclear DNA is organized in chromosomes.
- There are 22 pairs of autosomal chromosomes.
- There is one pair of sexual chromosomes; females are XX and males are XY.
- Chromosomes in somatic cells are in diploid state containing two sets of chromosomes, but in gametes, such as sperm or egg, they are in haploid state containing only one set of chromosomes.
- During conception, the union of the sperm and egg produces a zygote that is diploid again.
- This is why half of our genetic material comes from our father and the other half from our mother.
- In somatic cells, cell division, known as mitosis, produces daughter cells whose genetic information is identical to each other and to the parent cell.
- Cell division in gametes or sex cells is called meiosis.
- In meiosis, two consecutive divisions result in four daughter cells in haploid state.

Part 2 Organization of the DNA

- DNA is composed of two different regions referred to as coding and non-coding.
- The coding regions are known as genes, and they are responsible for the synthesis of proteins.
- Genes consist of exons (coding), and introns (non-coding) and they make up only 5% of the human genome.
- The remaining non-coding DNA, since no function is associated to protein synthesis, is called “junk” DNA.
- However, this non-coding region possesses a regulatory function.

Part 2 Organization of the DNA

- All the markers used in forensic DNA identification are located in non-coding regions either within or between genes.
- The chromosomal location of a gene in an intron is called locus.
- Usually, pairs of chromosomes are described as homologous.
- Homologous chromosomes have the same size and contain the same genetic structure.
- A copy of each gene is located at the same position on each chromosome of the homologous pair.
- The alternative possibilities for a gene are defined as alleles.
- If the alleles at a locus are identical on homologous chromosomes, they are defined as homozygous, and if the alleles are different at a specific locus, they are defined as heterozygous.
- The genotype is the characterization of the alleles present at a locus.

Part 3 Nomenclature for DNA Markers

- If the marker is located within a gene, the gene name is assigned to the marker.

  For example, the short tandem repeat (STR) marker TH01 is located in the first intron (01) of the human tyrosine hydroxylase gene (TH).

- If a marker is located outside a gene, then the following nomenclature should be followed: *D* (DNA) - *number of chromosome* – *S* (single copy) – *order of marker* was discovered and categorized for a particular chromosome.

  For example, the STR marker D7S820 is located in chromosome 7 and was the 820th locus described in chromosome 7.

  - D7S820
    - D: DNA
    - 7: chromosome 7
    - S: single copy sequence
    - 820: 820th locus described in chromosomes

Part 4 Physical Location in Chromosomes

- The chromosome basic structure includes the following:
  - A chromosome is composed of a central region, called centromere, whose function is to control the movement of the chromosome during cell division.
  - From the centromere, there are two arms that end with telomeres.
  - The short arm is designated *p* and the long arm *q*.
  - Human chromosomes are numbered from 1 to 22 based on their size.
  - Chromosome 1 is the largest and chromosome 22 is the smallest.
- There are two regions in chromosomes. One is transcriptionally active known as euchromatin and the other is inactive known as heterochromatin. One example of heterochromatin is the centromere.

Part 4 Physical Location in Chromosomes

- Chromosome structure can be visualized under a light microscope after staining with different dyes, which results in a banding pattern.
- A gene or a DNA marker can be mapped to a chromosome location.
- Bands can be located either in the long arm, q, or in the short arm, p.
- The band numbers increase outward from the centromere to the telomeres in both arms.

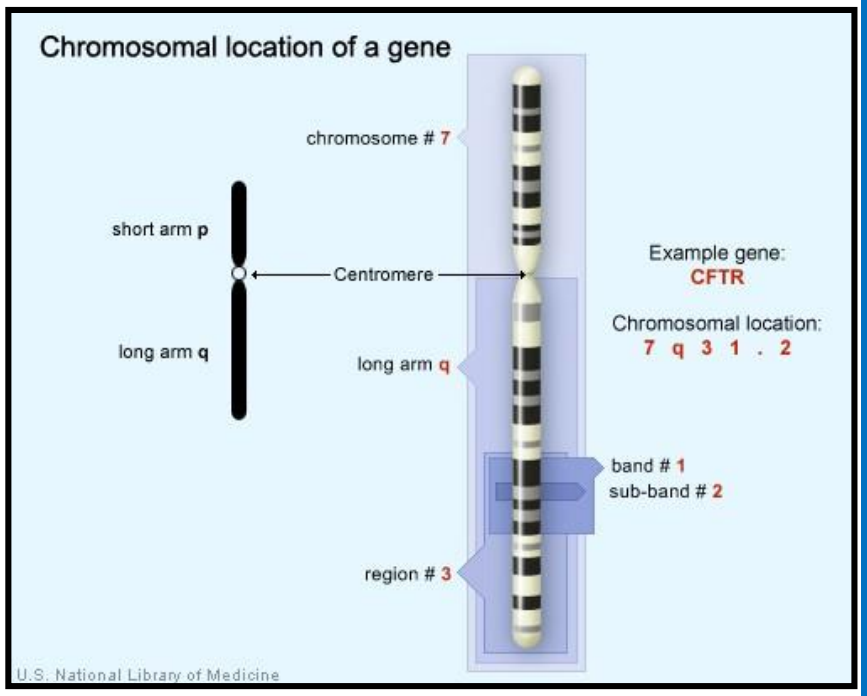


Figure 9
Basic chromosome structure and nomenclature

Part 5 Variation in Population

- The vast majority of DNA, around 99.7 percent, is identical among individuals; only 0.3 percent differs among people and makes us unique individuals.
- These variable regions were chosen for human identity testing.
- There are two forms of variation at the DNA level known as sequence polymorphisms and length polymorphisms.

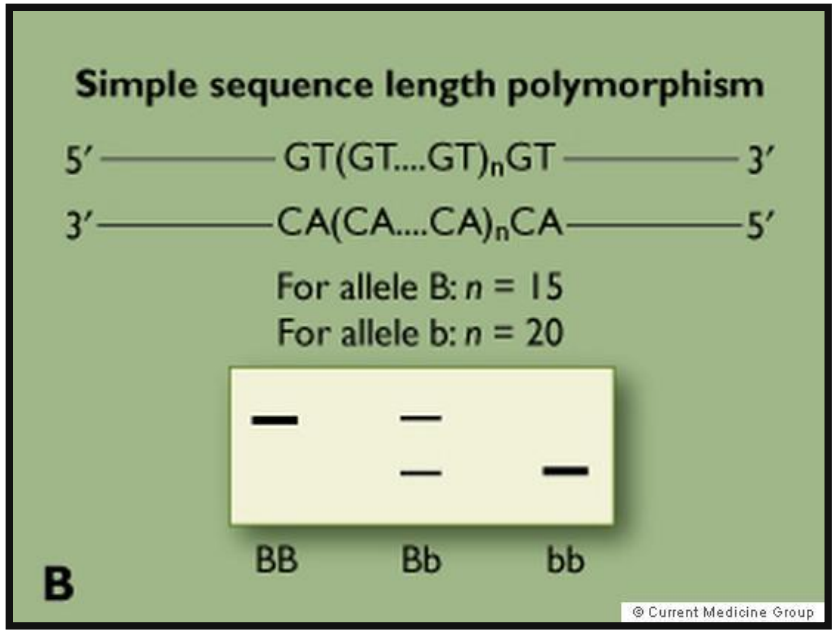


Figure 10
Length polymorphism

Part 5 Variation in Population

- The genotype is an indication of the allele state.
- For example, if one sample contains two alleles, one with 10 repeating units and the other with 12 repeating units, the genotype will be “10, 12.”
- If more DNA markers are used to analyze a sample, the chance that two individuals will have the same genotypes will be lower.
- In the same way, if there are matching samples, the more markers analyzed will add more confidence in connecting these two samples as being from the same individual.

Part 5 Variation in Population

- If the markers (loci) were inherited independently, then it is possible to multiply every genotype frequency to obtain the total DNA profile frequency. This is known as the product rule.
- Certain regions of the DNA are selected for analysis.
- The variability observed at these regions is then used to exclude or include samples.
- If there is an inclusion or a match, a virtual certainty is calculated to demonstrate that the probability of a coincidental match is extremely small.

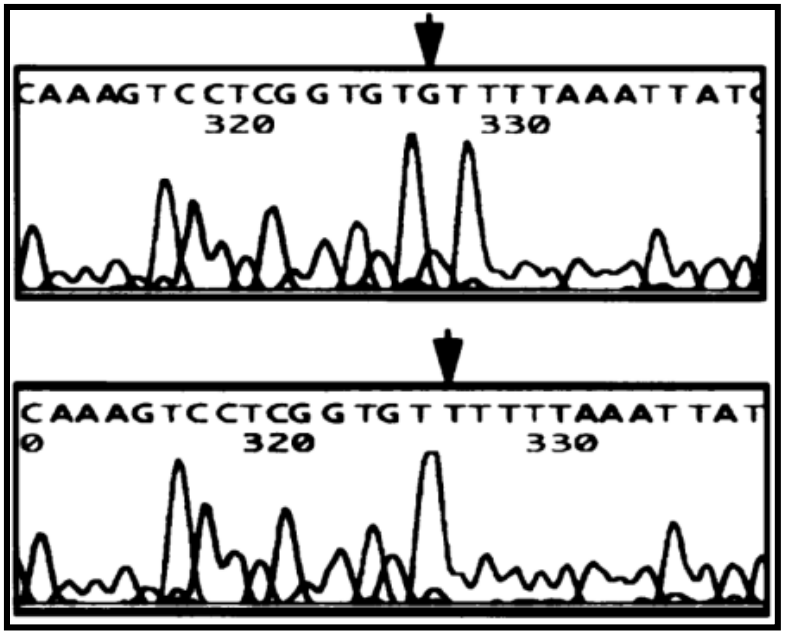


Figure 11
Sequence polymorphism

Part 5 Variation in Population

- If more DNA markers are used, the probability of this random match will become close to zero.
- A vast genetic variability exists in human population.
- In order to understand how the numbers of alleles at a particular locus impacts the variability, we will analyze the variability of the ABO blood group.
- If we have only three alleles, A, B, and O, there are six possible genotypes: three homozygotes (AA, BB, and OO) and three heterozygotes (AO, BO, AB). However, only four genotypes are expressed at the phenotype level since AO and AA and BO and BB are equivalent.

Part 5 Variation in Population

- Genetic markers contain large numbers of alleles, resulting in a larger number of possible genotypes.
- For example, a marker with 10 alleles would exhibit 55 possible genotypes and a marker with 20 alleles would give 210 possible genotypes.
- The use of 10 markers with 10 alleles each will produce more than 2.5×10^{17} possible genotypes.

Part 6 Methods to Measure DNA Variability

- An individual DNA profile can be obtained from low amounts of DNA in different biological tissues like blood, saliva, hair, or semen.
- Since all the cells in the human body possess the same DNA, any tissue collected from an individual will provide the same genetic information.
- Initially, RFLP methods were the methods of choice for human identification.
- Those methods were replaced by PCR-based techniques that allow analysts to work with much lower amounts of DNA and even with degraded samples (typical forensic samples).

Part 6 Methods to Measure DNA Variability

- The selected markers for human identification are the STRs because they are PCR-based; they can work with low quantities of DNA and degraded samples.
- STR systems can be easily automated and are very sensitive due to the use of fluorescent detection.
- Their power of discrimination is very high, and due to the nature of the discrete alleles, the information can be easily standardized between laboratories and allow the use of DNA databases.

End of Module 7A

Forensic Science Teacher Professional Development