Chapter 7 DNA Fingerprinting

By the end of this chapter you will be able to:

• explain how crime scene evidence is collected and processed to obtain DNA
• describe how radioactive probes are used in DNA fingerprinting
• explain how DNA evidence is compared for matching
• explain how to use DNA fingerprinting to identify DNA from a parent, child, other relative, or a non-related individual

Introduction

Since the 1980's, DNA has been used to investigate crimes, establish paternity, and identify victims of war and large scale disasters.

• DNA is unique (individual evidence). It has been used to identify a victim even when no body is found
• DNA is used to identify victims of plane crashes and hurricanes

History of Biological Evidence in Forensics

• Biological evidence (blood, saliva, urine, semen, hair) is used for identification purposes
• Bio evidence is also examined for the presence of inherited traits such as blood type or enzyme variants
• Most lab techniques used in forensics were originally developed for other purposes such as medical diagnosis or treatment
• Blood typing was first used to make transfusions safe
• It can eliminate suspects, determine whether blood at a crime scene belongs to one person or multiple people, and give paternity results

The Function and Structure of DNA

• DNA holds all of the information and instructions needed for a cell to make proteins and replicate
• DNA molecules make up chromosome structures and are found in the nucleus of cells in the human body.
• Under the protective histone packaging, is 2 strands, and each DNA strand is tightly coiled around protein molecules and itself
• Know double helix, Watson & Crick
• Sides of the "ladder" are the backbone, made up of alternating sugar and phosphate molecules, the "rungs" are nitrogenous bases

• Bases: adenine (A), cytosine (C), guanine (G), and thymine (T)
• Each base is bonded to a sugar in the backbone and forms weak hydrogen bonds with a specific base on the other strand (A-T and C-G)
• Complementary - CGTATT bonds with GCATAA
• 23 pairs (46 total) of chromosomes in humans (except sex cells which have 23 total). One of each pair from mom and one of each pair from dad
The Function and Structure of DNA

- DNA in chromosomes is called nuclear DNA and is virtually identical in all cells of the human body
- Mitochondrial DNA is in a circular loop and, unlike nuclear DNA, is inherited only from the mother.
- Mitochondrial DNA is passed onto the offspring in the cytoplasm of the egg cell; none of the mitochondria come from the sperm cell

• Each chromosome has many genes
• Genes - DNA sequences with instructions that determine inherited characteristics or traits, such as blood type
• Genes also make RNA
• Allele - one of two or more alternative forms of a gene. One allele might code for normal hemoglobin and another codes for abnormal hemoglobin
• Homozygous dominant, heterozygous, or homozygous recessive
• One allele comes from mom and one from dad

For each topic, research both sides of the argument. Use internet searches, your own opinion, etc to back up the pro and con sides. We will debate these topics in class. You must have several points for each side of each topic

Debate topics:
1. Should the government have access to everyone’s DNA?
2. Should the DNA of relatives of convicted criminals be available?
3. Should we screen everyone’s DNA at birth to identify possible health issues?
4. Should we be allowed to genetically alter plants and animals to make food more desirable for human consumption?

DNA Identification

- Most of human DNA is the same, but Junk DNA contains many of the unique patterns of repeated base sequences that identify individuals.
- Differences in DNA sequences are called polymorphisms.
- In 1984, Dr. Alec Jeffreys, developed a technique for isolating and analyzing these variable areas.
- DNA Fingerprinting appears as a pattern of bands on X-ray film. Patterns can be used to identify individuals.
- Determine if 2 or more samples are from same person, related individuals, or unrelated individuals

DNA Identification

- Genome - total amount of DNA in a cell contained in chromosomes and mitochondria
- 3 billion base pairs in chromosomes
- Encoded DNA(exons) - used to make proteins
- Uncoded (introns) - doesn’t produce protein or RNA
- Nuclear DNA roughly 23,688 encoded genes, each averaging 3000 base pairs. Less than 1.5% of genome
- Other 98.5% is noncoding. May be involved in gene regulation or splicing. Rest is sometimes called “junk DNA” - new information about its function is being examined

The number of copies of the same repeated base sequence in DNA varies among individuals.
- Forensic Scientists focus on 2 types of repeating DNA sequences in the noncoding sections of DNA known as Variable Numbers of Tandem Repeats (VNTRs) and Short Tandem Repeats (STRs)
VNTRs
- Within junk DNA, sequences of DNA are repeated multiple times.
- The repeated base sequence CATACAGAC might be repeated 3 times in one person and 7 in another
- Because the number of repeats varies from one person to another, these multiple tandem repeats are known as variable number of tandem repeats
- VNTRs range from 9 to 80 bases in length.

STR
- These usually are only 2-5 bases in length and are becoming the preferred sequences for analysis.
- The high degree of polymorphisms in STRs result from the different number of copies of the repeat element that occur in a population
- It is preferred because of its accuracy and because small and partially degraded DNA samples may be analyzed to identify individuals
- The longer length of VNTRs makes it more difficult to separate the sequences

DNA Profile
- VNTR and STR data are analyzed for (a) tissue matching and (b) inheritance matching.
- Tissue matching - 2 samples that have the same band pattern are from the same person (as in a match from a crime scene sample and a suspect)
- Inheritance matching - the matching bands must follow the rules for inheritance; each band in a child’s DNA profile must be present in at least one parent

Population genetics
- Population genetics - study of variation in genes among groups of individuals.
- Proportion of people who have a particular characteristic is determined by the proportion of alleles for these traits in the population
  - Ex: alleles that code for blue eyes are rare among the Asian population

DNA Databases
- Extensive studies are done to determine the percentage of the populations with a particular allele
- The data is entered into databases
- Calculations can be made based on these groups to determine the probability a random person would have the same alternative form of a gene (an allele) as (a) a suspect in a crime or (b) an alleged father in a paternity case.

Sources of DNA
- A perpetrator may leave biological evidence, such as saliva or blood, at a crime scene.
- This individual evidence is capable of identifying a specific person. Ex: saliva can be collected from an envelope, toothbrush or bite wound
- But a small amount of biological evidence might be considered only trace evidence, and it may be consumed during forensic testing.
Sources of DNA

- In 1993, however, the polymerase chain reaction (PCR) technique was invented. It generates multiple identical copies from trace amounts of DNA evidence.
- Dr. Kary Mullis invented PCR and shared the Noble Prize for it.
- Can make billions of copies from small amounts of DNA in a few hours.
- The copies are then tested so original evidence can be preserved.

Collection and preservation of DNA

- Must be careful not to contaminate DNA.
- Use disposable gloves and collection instruments.
- Avoid physical contact, talking, sneezing, and coughing in the evidence area. Avoid touching face.
- Air-dry evidence and put it into new paper bags or envelopes.
- If evidence cannot be dried, freeze it.
- Keep evidence cool and dry during transportation and storage to avoid mold growth.

Preparing DNA Samples for Fingerprinting

- DNA is mixed with special enzymes (restriction).
- The enzymes cut apart the DNA in specific places forming different sized fragments.
- The DNA is loaded into the chambers found on an agarose gel.
- An electric current is passed through the gel separating the fragments by size.
- Larger fragments stay near top, smaller ones migrate toward bottom.

Step 1: Extraction

- Cells are isolated from biological evidence such as blood, saliva, urine, semen, and hair.
- The cells then are disrupted to release the DNA from cell components.
- Once released, the DNA can be extracted from the cell nucleus.

Step 2: Restriction Fragments

- Restriction enzymes - recognize a unique pattern of DNA bases (restriction sites) and will cut the DNA at that specific location forming fragments.
- These enzymes are often produced and used by bacteria to defend themselves from invading viruses.
- Each enzyme cuts a different site.
- Within some of the fragments are the VNTR sequences.
Step 3: Amplification

- With some VNTR analysis, polymerase chain reaction (PCR) can be used to amplify the DNA that contains the VNTRs.
- In STR profiles, restriction enzymes are unnecessary; PCR allows the amplification of the strands with STR sequences.

Step 4: Electrophoresis

- DNA samples are placed in gels through which electronic currents are passed.
- The cut DNA fragments line up in bands along the length of each gel according to their length (long stay at top near - and smaller ones migrate toward + end).
- Each sample has its own lane and a control lane is used with known fragment lengths (called DNA Ladder or Standard DNA)
- complete when DNA fragments have migrated through the gel

Step 4: Electrophoresis

- If not amplified by PCR, the fragments need to be enhanced by radioactive probes
- Probes bind to specific sequences
- To do this, the fragments on the gel are transferred to a membrane in a process called Southern blotting

Probes

- It is impossible to look at all fragments produced from an individual's DNA (it would look like a smear instead of bands)
- DNA probes are used to identify the unique sequences in a person's DNA creating the bands.
- If PCR is used, only fragments that were amplified would show up as bands, so there is no smear
- Fluorescent stain, in the liquid that the gel is in, inserts itself into the gaps of the DNA double helix and an ultraviolet light is used to make the bands visible

Probes

- Different DNA probes are made up of different synthetic sequences of DNA bases complimentary to a small portion of the DNA strand.
- The probe binds to complimentary bases in the strand.
- Ex: if DNA code is AAGCTTA, then the radioactive probe is TTCGAAAT. The probe will attach everywhere the DNA code is found on the fragment
- Depending on the probe, the bands may be viewed under UV light, X-ray (autoradiograph), etc
- In most criminal cases, 6-8 probes are used.

Paternity

- If the two alleles that the child inherits from the parents are different, than two bands will appear.
- If the child inherits the same allele for a gene from each parent, then a single band will appear (see yellow above)
The greater number of probes used, the greater the accuracy. The position of the bands and widths are significant in matching samples of DNA and all the bands must match exactly.

The U.S. has Combined DNA Index System (CODIS) - an electronic database of DNA profiles. Every state maintains DNA index of people convicted of certain crimes (rape, murder, or child abuse). Similar to AIFIS, DNA at crime scene can be compared to CODIS to search for a match. DNA fingerprinting can (a) match crime scene DNA with a suspect, (b) determine maternity, paternity, or match to another relative, (c) eliminate a suspect, (d) free falsely imprisoned individual, & (e) ID human remains.