DNA Analysis and its impact on Judicial Decision Making

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INTRODUCTION

- DNA -> Deoxyribonucleic acid.
- Located in Chromosomes in the nucleus of cells
- Tightly packed genetic information
- A polymer made up of nucleotides
- Genes – options of DNA that code for traits
- and functions- 35000 genes in human body.
- About 100 million nucleotide in one DNA molecule

- **Nucleotides** made up of three parts

  Sugar (deoxyribose)

  Phosphates

  Bases – Purines :- A – Adenine

  G – Guanine

  Pyrimidines :- T – Thymine

  C – Cytosine

They bind with specificity :- **A-T & G –C**

Each base contain Nitrogen and so they are sometimes referred to as **Nitrogenous** bases

- Importance of Base pairs :-
- Can predict sequence of one strand based on the sequence of the other
- Responsible for Replication and Transcription
- Repair damaged DNA

- Specific sequence of nucleotides of all human beings is 99.9% - the same
- It is that 0.1 % difference that makes each individual unique

- The sequence codes for amino acids -> proteins
- If not ordered correctly – Nonsense codon – aberration – mutation

- Can you match the sequence :-

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

Where is DNA found

- Blood
- Semen
- Skin
- Cells
- Tissues
- Organs
- Muscles
- Brain cells
- Bone

Teeth
Hair
Saliva
Mucus
Perspiration
Fingernails
Urine
Faeces
etc

Forensic Use of DNA

- Identify potential suspects
- Exonerate persons wrongly accused of crime
- Identify crime & criminal investigations
- Identify Catastrophe and natural disaster victims
- Identify persons who are otherwise difficult to establish – decomposed, completely burnt, skeletonized, mutilated
- To solve any dispute of identification
- Establish paternity and other relationship
- Detect mutated genes, genome mapping, genetic disease analysis

DNA Analysis

- Emerged in mid 1980 by Alec Jeffreys, an English geneticist
- Forensic scientist scan 13 DNA regions or loci and create a DNA profile for that person :- DNA Fingerprint
- In criminal cases , samples are collected from the crime scene , and from one or more suspects, extracting DNA and analyzing it for a set of specific DNA markers
- Markers are found in DNA by making small pieces of DNA (Probes) that will seek out and bind to a complementary DNA sequence in the sample.
- More probers used , greater the odds for a unique pattern and definite match.
- 4 – 6 probes recommended.

Matching

- The DNA profile compared -> to see whether the suspect sample matches the evidence sample
- If no match-> suspect not the accused
- If match found -> suspect is the accused

- Portions must be large enough to exclude biasness and increase specificity – that it is not random

CODIS

- Combined DNA Index System
- A program which consists of many databases that have DNA profiles useful for the criminal justice system
- NDIS (National DNA Index System) , part of CODIS – contains DNA profile at a national , state and local level and is accessible to the law enforcement all over the country

DNA as Evidence

- Easily contaminated Misleading result
- Many guidelines for handling of samples
- Degradation
- Monitor collection and Storage
- Successful extraction by PCR technology

- DNA testing process comprised of 4 steps :-

  - Extraction
  - Quantitation
  - Amplification
  - Capillary Electrophoresis

Extraction

- By Manual methods
- By Commercially available Kits
- By Direct Fine Needle Aspiration Cytology
- From Frozen Section of tissues
- From Formalin Fixed paraffin embedded tissues

- Tool used – Centrifuge- Separation – Precipitate-Wash by ethanol/ Isopropanol or a Gel Box in agarose gel with an electric charge

Quantification

- By NanoDrop Spectrophotometer – measure level of UV light absorbed by bases.
- Amount of Light absorbed is proportional to the concentration of DNA in the sample.
- At 260 nm absorbance to estimate nucleic acid concentration
- At 260/325 nm absorption ratios – to determine DNA purity & presence of contaminants in biological samples

Amplification

- By Polymerase Chain Reaction (PCR)
- Technique involving enzymatic amplification of nucleic acid sequences via repeated cycles of denaturation oligonucleotide annealing, & DNA polymerase extension.
- In vitro technique

Capillary Electrophoresis

- High output separation method commonly used for DNA analysis owing to rapid analysis times and small sample volumes
- Performed in a sub-millimeter tube, called a capillary tube, which contains a flowing electrolyte solution.
- The sample is injected into the capillary and an electric field applied

STR Analysis

- Short tandem repeat analysis – 1 to 10 base repeats
- Amount required is 1 microgram to 10 nanogram
- The most common type of DNA profiling for criminal cases & other Forensic uses
- Y-STR Analysis is able to detect the presence of miniscule amounts of MALE DNA of one or multiple donors & resulting genetic profiles can be compared with known reference samples

RFLP

- Restriction Fragment Length Polymorphism
- Difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples in question with specific restriction endonucleases
- Cleaves double stranded DNA a specific 4-8 nucleotide long palindromic sequence
- The enzyme RE has specific sites on DNA- cuts it into fragments
- Different size with specific desired sized fragments produced

- Then comes Gel electrophoresis
- The digested fragments are run into gel electrophoresis to separate the fragments on basis of length, size or molecular weight
- Denaturation :- The gel is placed in NaOH solution to produce single stranded DNA
- Blotting (Southern type) :- The single stranded DNA then transferred into charge membrane i.e. Nitrocellulose paper by process called capillary or electro- blotting
- The nitrocellulose transferred paper fixed by autoclaving , then blocked by using bovine serum albumin – to prevent binding of the labeled probe nonspecifically to the charged membrane

- Hybridization & Visualization :-

➢ The labeled RFLP probe is hybridized with DNA on the nitrocellulose paper

➢The RFLP probes are complimentary as well as labeled with radioactive isotopes – they form color bands under visualization by autoradiography.

Laws related to DNA Technology

- Several Laws – vary from state to state
- DNA can last for many years statutes are limited at different places
- Limits the duration between crime and conviction
- Statues becoming obsolete due to the reliability of properly stored DNA samples.
- DNA databases lead to convictions – laws to ensure success
- Some convicted offenders required for databases

Equipments for DNA analysis

- DNA Thermocycler - used to amplify segments of DNA via polymers, can be programmed o carry out heating & cooling of samples over a number of cycles
- ABI 300 genetic Analyzer – used for STR genotyping
- Thermostable DNA polymerase – eg Taq polymerase which is not deactivated by heating to 95 degree C

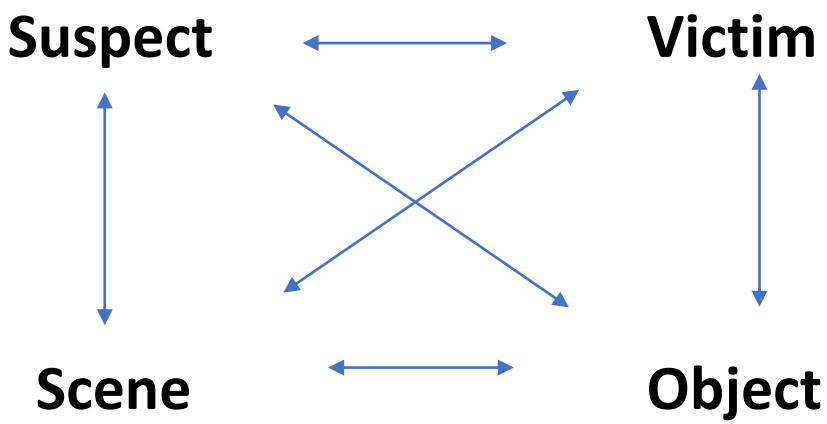
DNA Techniques

- RFLP – restriction fragment length polymorphism
- Blood stain -> DNA extracted from bloodstain -> Restriction enzyme cleavage of DNA -> Fragments of DNA separated by Electrophoresis -> Transfer of DNA fragments to a membrane (Southern Blot)->Radioactive DNA probe binds to specific DNA fragments.

Comparison

- **Questioned sequence to Known Sequence**

	Suspect #1	**Suspect #2**
Questioned	GCATATTGCGCCTA	GCATATTGCGCCTA
Known	GCACATTACGTCTA	GCATATTGCGCCTA
	***Exclusion**	***Inclusion**
	No match	**Match**



Brief History of DNA Matching

- 1970s - HLA matching
- HLA inherited from both parent .Used for paternity testing, biological relationships, High exclusion rate 80%
- 1980s- DNA testing using RFLP technique
- 1990s – DNA testing using PCR testing
- 2007s and beyond – Y-STR & mDNA used male & female line respectively

Chain of Custody

- To see DNA samples not contaminated
- Proper paperwork required for court proceedings
- DNA Collected by third party laboratory professionals
- Gloves worn, caution observed
- Collected, sealed, packed
- Receiving laboratory will first check if collect samples properly packed before proceeding to work on DNA
- Apart from Exclusion & Inclusion matches, Inconclusive results – from possible contamination , very small amount, degraded – cannot produce accurate DNA profile

Exoneration - Lacunae

- Previously convicted for a crime – found to be innocent later on
- DNA testing – most common method of post – conviction exoneration of wrongfully accused
- Most of the exonerated persons are wrongfully convicted due to eyewitness misidentification (Innocent project)
- Most of the exonerated persons were convicted of rape and assault , convicted by judiciary, but exonerated by Science (Case studies in the use of DNA evidence to establish innocence after trial , 1996)

The first convicted defendant to be exonerated by DNA evidence testing in 1989, Virginia

- Wrongfully convicted defendant to be exonerated by DNA evidence testing in 1989, Virginia
- Erroneously convicted of Sexual assault followed by hanging a woman in her Arlington County, Virginia home, thereby sentenced to 35 years in prison
- Conviction on the basis of two witnesses – placed him near the scene of crime - eyewitness misidentification – he pleaded guilty because he dreamt that he had committed the crime
- Three laboratories conducted DNA testing on this case – found that the pubic hair from the scene of crime inconclusive with Vasquez's
- Exonerated in 1989 after having served 5 years of his sentence
- Who is at fault ? Who will give him back his 5 years ?

Controversy

- Justice for all Act –once reserved for those convicted of violent offences , but now for anyone charged with a crime
- Police given authority to collect DNA from any person arrested on suspicion of a recordable offence , not charged, nor convicted
- Innocent peoples' profiles on database to get results – but becomes an automatic suspect for any crime in future- undermines principle of presumptive innocence
- Listed databases – jeopardize employment , foreign travel, but can be used for genetic correlates of criminal behavior

Is DNA Database – a violation of Privacy

THANK YOU