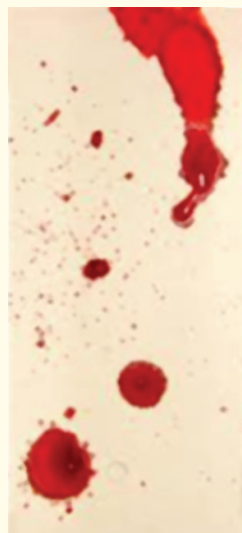
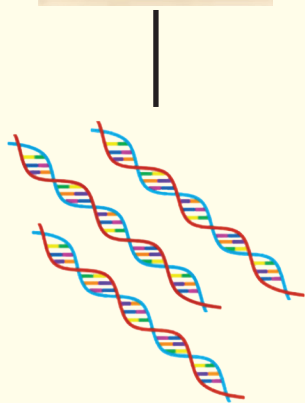


DNA Phenotyping Future of Forensic DNA Typing

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(Biological Sample from crime scene)



(Extraction of DNA)



(Analysis of SNP sequences using NGS)



(Prediction of phenotypes)

Technology of prediction of human phenotype from the crime scene samples

DNA fingerprinting analysis relies on the comparison of DNA profiles between the control or reference sample and the questioned sample. Short Tandem Repeat (STR) markers present on autosomes, X and/or Y chromosomes are amplified using multiplex PCR technique. The amplified DNA fragments are subjected to capillary electrophoresis to give rise to a DNA profile.

After generation of the DNA profiles they were analyzed and the results are matched between the two samples. Based on a match or mismatch various types of cases such as paternity dispute, identification of mutilated bodies, murder or sexual assaults are solved with great fidelity.

Being a technique of comparison, the current day DNA fingerprinting relies solely on the availability of reference or

control sample. The control sample may include biological sample of the putative relatives like father, mother, brothers, sisters, wife and child for identification purpose. Similarly, a reference sample from the suspect is essential for deciphering a case of murder or sexual assault.

This is a huge challenge for the investigating agency to arrange the reference or control sample for DNA examination mostly in cases of blind murder or other cases where the suspect is not known or the relatives of a mutilated body are found. In such cases, it becomes the tiring job of a DNA scientist to examine 'n' number of samples before drawing a conclusion.

The first cases in history to be solved by DNA fingerprinting screened more than 1000 samples of villagers

to find the culprit in a serial rape and murder case of Lynda Mann and Dawn Ashworth. To deal with this problem, many countries have their own DNA databases, and a search of an unknown DNA profile in the database can give rise to a hit. But it may not always work either due to unavailability of that profile in the database or when the culprit is an outsider.

Some countries like India do not have a database of their own yet. In such cases, it becomes immensely difficult to draw a conclusion from the DNA fingerprinting examination either due to unavailability of the reference samples or mismatch in the database search. To deal with this problem, the advanced technology of DNA phenotyping comes to the rescue where the phenotype or external features of an individual can be predicted by analysis of DNA generated from the crime scene.

DNA Phenotyping

When genetic variation occurs in the regions that code for visible characteristic of human beings, a distinct phenotype is observed. Some of these genetic traits such as hair colour, eye colour, skin colour and face morphology are highly heritable and are not influenced greatly by the environmental factors. Thus, using the genetic information available on the DNA, these phenotypes can be predicted with a high degree of confidence.

A study in 2018 deciphered 15 genes responsible for unique facial characteristics of an individual. These genes determine the cheekbone prominence, distance between eyes, chin shape and shape and length of nose, eye colour, hair colour, skin colour, height and baldness.

DNA phenotyping technique is capable of predicting the external appearance of the suspect irrespective of the availability or unavailability of the suspect's DNA in the database. It deciphers the SNPs of the genome that code differently for different people. Single Nucleotide Polymorphism (SNP) is considered to be the simplest form of genetic variation where change occurs in the nucleotide sequence due to either transition (A with G or C with T) or transversion (A with T or C with G) substitution mutation of nucleotides. They are found abundantly in the genome as one in 1000 bp. These SNPs are either silent substitutions and do not affect the coded protein or affect different phenotypic traits of the individuals i.e. eye colour, hair colour, skin colour, height and baldness.

In this technique, the DNA is isolated from the crime scene. The sequences of millions of pieces of DNA from a small amount of DNA are generated using Next Generation Sequencing (NGS) to get the SNP sequences. The SNP sequences and their corresponding phenotypic features are analyzed. The paired SNP genotypes and phenotypes from thousands of individuals are generated to form a genotype and phenotype (GaP) database. With the help of various models, the obtained SNP sequences from the crime scene are translated to the physical appearance traits with greatest likelihood.

Though DNA phenotyping is a new technique, it has been widely used in criminal investigation and has solved many cases by providing leads, narrowing down to a pool of

suspects and providing solutions to many old and recent cases in history.

In May 2016, a missing person's report was filed for 25-year-old Rhonda Chantay Blankinship in Texas. After two days, the dead body of Blankinship was found abandoned in an area 5 miles from her home. Autopsy confirmed that she was sexually assaulted prior to her murder. When DNA test was conducted, a male DNA profile was detected, but no hits could be produced in the DNA database. When DNA phenotyping technique was applied and suspect's possible sketch was released, the investigating agency received multiple leads in this case and finally, police could successfully identify Ryan Derek Riggs, the alleged boyfriend of Blankinship as the culprit in this case.

In June 2017, a badly decomposed remains of a human body was found in Glen Burnie, MD. During autopsy the skeleton was predicted to be of a 20-year-old female. However, the investigation team failed to identify the deceased due to non-availability of DNA samples of close relatives. Finally, DNA phenotyping technique was applied on the DNA obtained from the human skeleton and African-American ancestry of the individual with brown skin, brown eyes, black hair, and no freckles were predicted for the deceased.

Immediately the investigating agency found inputs regarding similarities between the predicted physical appearance and Shaquana Caldwell aged 26 years and further examination revealed the true identity of the deceased.

Other Applications of DNA Phenotyping

In addition to predicting phenotype of an individual, these Phenotype Information Markers (PIMs) are useful in molecular diagnosis of various diseases and detecting human pathogens. More than 3000 monogenic diseases can be diagnosed using these genetic markers at either pre- or post-natal stage such as Down's (Trisomy 21), Turner (Monosomy X without another sex chromosome), Klinefelter (Disomy X with the presence of Y), Patau (Trisomy 13), Edwards (Trisomy 18) syndromes. Other genetic diseases that can be detected by these markers at an early stage include achondroplasia, cystic fibrosis, Sickle cell anaemia, Huntington's disease, Tay-Sachs disease and many others. Other diseases such as cancers, obesity, hypertriglyceridemia, schizophrenia, autism, ADHD and Alzheimer's disease can also be detected using these genetic markers.

Unlike the traditional methods of pathogen tracking in human body like immunological methods, microscopy, or direct isolation, genetic markers can be widely used for detection of many pathogenic organisms such as *Mycobacterium tuberculosis*, Human Papillomavirus (HPV), *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, avian influenza virus and influenza A (H1N1) virus, Human Immunodeficiency Virus (HIV), measles, hepatitis A, B, and C and other pathogenic organisms.

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