Review

Diagnostic Revolution in Forensic DNA Analysis - A Review

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Abstract

Forensic DNA or DNA profiles has played major role in criminal justice system. Newer development in the field of nanotechnology has been of increasing interest in recent years. This paper reviews the evolution of DNA fingerprinting in the field of forensics to a point where trace amount of 'Junk DNA' can be used in 'Point of Care' diagnostics, thus increasing the practice of forensic odontology. A point of care device, microfluidics (lab on a chip) is becoming a cutting edge diagnostic technology in forensic analysis as it is designed to access advanced laboratory facilities within a bio-chip.

Key Words: DNA fingerprinting, forensic DNA, microfluidics, lab on chip, point of care diagnostics.

Introduction

Forensics play a main role in identifying an individual. There are many circumstances where an individual identity is of primary concern as in road accidents, mass disaster, crime scenes, sexual assaults etc.

Various approaches such as lip prints, bite marks, DNA profiling have been made to identify individual.(1) Of all traditional methods of identification. DNA profiling has come a long way from conventional fingerprints and has revolutionised the aspect of forensic odontology.(2) DNA analysis is considered greatest forensic tool since no one can alter their DNA sequence and it is hard to prevent leaving one's DNA at the scene.

STR (Short Tandem Repeats) are the simple sequences which repeats on DNA. The number of repeats in STR is highly variable among individuals. STR has many benefits such as it can be easy amplifiable, smaller size, easily separable, lower mutation rate etc. Therefore, with techniques like STR, we can characterise individual at fundamental level of DNA and variation between at this level can be used to discriminate between individuals.

Microfluidic forensic DNA analysis system is relatively new field which is aiming at truly automated chip to deliver DNA fingerprinting in minutes contrary to conventional DNA profiling which is time consuming and require technical expertise.

This review highlights the technique and concept that are vital to the successful outcome of the investigation and thus increase the practise of forensic odontology at ease.

DNA Fingerprinting

DNA testing is now an essential part of crime laboratory's armamentarium as they provide the best biometric information yielding identity. "Every contact leaves a trace". DNA typing was introduced into forensic science in the mid 1980's arising from discoveries made in biomedical research. This has become such an effective tool in forensic biology that almost all of the traditional techniques have fallen into disuse.

The first hours of investigation is considered the golden hour of investigation. For these reasons, there is strong need for relevant information becoming available as quickly as possible. But conventional forensic DNA analysis takes time and requires technical expertise. There are three to four different steps that a sample has to go through and minimum of three instruments are involved before any data is generated, each step taking at least 30 minutes to run. There are situations in which it is important to quickly and positively identify an individual. In this regard, systems utilised must be fast, portable and easy to maintain.

Three generations of DNA testing have been discovered over past decade. They include Restriction Fragment Length Polymorphisms (RFLP). Ray white, an American geneticist, identified regions of DNA that did not code for proteins but were highly variable between individuals. The strands of DNA were cut at specific location based on size and DNA fragments of defined lengths were produced, calling the variations restriction fragment length polymorphisms.(3) DNA readout was in the form of gel electrophoresis (Autoradiogram).

With this discovery, methods for mapping human genome were described and found that RFLP could be used to develop patterns of restricted DNA that were more or less specific to an individual.

Process is extremely laborious and time consuming. Relatively large amount of sample is required and takes 2 – 3 months to generate results.

DQ ALPHA(4)

This is the first PCR based test. This test uses test strips, checks for the presence or absence of blue dots in the strips. The intensity of blue dots differ and each

intensity of this blue denotes the presence a particular type of DNA sample.

Even though this technique has got some advantages like faster results (around half a day), relatively less sample required and much sensitive method, the discriminative power has not been satisfactory.

Short Tandem Repeats (STR)

Automated STR test is the 3rd generation DNA test. Eukaryote genome is largely made of coding and noncoding regions. The DNA in the noncoding regions referred to as 'Junk DNA' containing certain untranscribable or untranslatable DNA base sequences also appears to be useful. These base sequences shows sequence repeats and are termed satellite DNA. If the Repeats range between 2- 4 nucleotide sequence, it is termed microsatellite DNA or Short tandem Repeats (STR). Short tandem repeats which are sometimes referred to as microsatellite or simple sequence repeats, are accordion like stretches of DNA containing core repeat units of between 2 and 7 nucleotides in length that are tandemly repeated from approximately a half dozen to several dozen times.[5] These repeat sequence varies from person to person. These nucleotide variations help in making DNA fingerprinting of an individual and also understand DNA polymorphisms.

STR analysis is the current method of choice in forensic DNA analysis as it yields results that are nearly equivalent to individualisation. It is worth noting that these core STR loci occur in between genes in which a high degree of variability is tolerated and are thus not directly responsible for physical traits such as hair color or eye color or genetic diseases.[5]

Although human genome contains thousands upon thousands of STR markers, only a small core set of loci have been selected for use in forensic DNA and human identity testing. Millions of STR profiles are generated worldwide performing various forms of human identity testing, including DNA data basing, parentage testing. The conversion of sized DNA fragments to genotypes is the standardization between all forensic DNA laboratories for comparing data and is essential for laboratories utilising Combined DNA Index System (CODIS) database to compare profiles. Current forensic DNA typing kits uses a set of universal core loci established by Federal Bureau of Investigation (FBI) that have been catalogued in their CODIS database system. These universal 13 core loci contain highly polymorphic tetra nucleotide repeats that can be used to discriminate between individuals for forensic identification.

The short size and number of available STRs allowed scientists to amplify and analyse 3 or more loci simultaneously (multiplexing). Use of multiple loci enables a high power of discrimination in a single test without consuming much DNA.

STR are highly polymorphic ,require less sample material, much less than what can be seen through naked eye, generates very fast results, few hours to generate, discriminating power is high, had been initially discovered and was popular in 1990. PCR is widely used for STR profiling of DNA samples.

Other DNA tests include mitochondrial DNA test which is maternally inherited, sensitive, not discriminating and likely to be unique. Y–STR test is used for male DNA profile, when mixed samples are used.

In order to obtain results from the data present, various techniques have been used. They include

Polymerase Chain Reaction (PCR)

PCR is technique for replicating DNA in the lab (molecular xeroxing). In 1983, Kary Mullis developed the technique known as PCR which ultimately revolutionised molecular biology, including forensic DNA analysis.[6] Through PCR, forensic DNA analysis essentially became more rapid and sensitive.

Currently DNA sequencing and fragment sizing instruments are difficult to use and require highly trained operators. Although advancements to methods in forensic biology have streamlined the analysis process, the community still continues to research to develop faster methods.

Microfluidics or Lab on Chip (LOC)

One proposed alternative to lab analysis is the microfluidic total analysis system. A newer generation of point of care technology called lab on chip platform seeks to integrate and automate all the complexities of lab procedure into device, the size of computer chip. Ideally devices are used that can collect a sample from the crime scene, perform sample work up (Lysis and Extraction of DNA) and amplify and analyse the DNA.[7] The fully integrated system in development would essentially eliminate all manual processing by minimising the work to simply introducing a sample and a single button pressed. As a result, only minimal training could be required and any untrained personnel could operate it.

Microfluidics also lends itself well to automation, so researchers can make platforms that are very simple to use. All a person potentially has to do is take a sample, stick it into a machine, press the 'go' button, and wait for the instrument to spit out a DNA fingerprint. In many ways it is the same old chemistry but in a miniaturised form that now seamlessly integrates all

three or four of the processes onto one device and closes it off so that it is not susceptible to contamination.

An ideal microfluidic device should have ruggedization, should have capacity to handle various sample types and should have increased sensitivity and specificity superior to those performed conventionally using normal techniques and robotic instrumentation.

PCR steps uses the block thermocycler traditionally to cycle between the various temperatures, whereas researchers like Landers use infrared heating in microfluidics, which is much faster.[8] This shows that time saving comes in almost every step of the microfluidic process.

Microfluidic devices developed for point of care and clinical applications for cell lysis, sample work up, PCR and detection / analysers create opportunities which can also be applied within the forensic field. This self-containment means that human hands are taken out of the picture and reduces the potential for contamination, a crucial issue in forensic analysis.

The advantages of microfluidic DNA integrated device provides a degree of control over volumes, flow rates, surface area and composition and temperature control that cannot be obtained using conventional macro scale tools such as pipettes, centrifuge and mixers.[9] Single use biochips would minimise risk of contamination, need for cleaning and reprocessing of bio chips, makes it portable, can be carried out in resource limited areas, generate actional data in real time and cost effective. Secondly miniaturization allows for automation of parallel processes at high throughput and at a scale previously achievable only with large laboratory robots.

But the lack of awareness among health professionals like dentist, leads to its limited utilisation with subsequent lower production.

STR in Microfluidics

STR typing process is broadly used in molecular forensic analysis.[5] Conventional technique require experienced lab technicians to carry out the protocol. Although highly successful and reliable, current methodologies require 8-10 hours to complete under routine conditions, uses large sample volumes, costly reagents, and are labor-intensive.

The goal is to develop a rapid system capable of separations on short channel microfluidic devices with the required resolution needed for microsatellite analysis in forensic DNA samples.

To simplify the implementation of this process, it is desirable to use microfluidic technology and system engineering to build a platform that involves fully integrated disposal catridges which can be used by minimally trained operators and increase throughput capabilities with greater reproducibility of the test.[10]

The entire process from sample collection to obtaining a STR profile takes approximately 4 hrs. The instrument is loaded with a DNA processing cartridge which incorporates on-board pumps and valves which direct the delivery of sample and reagents to the various reaction chambers to allow DNA purification, amplification of the DNA by PCR. Following multiplex PCR amplification, DNA samples containing the length variant STR alleles are typically separated by capillary electrophoresis and genotyped by comparison to an allelic ladder supplied with the commercial kit.[5]

In order to overcome the need for quantification, a silica filter or beads can be used, which have a specific binding capacity to prevent an excess of DNA available for the amplification reaction. Utilizing a single, integrated and disposable microfluidic chip, the multi-step sample processing and analysis that consumes 8-10 hours for conventional forensic STR analysis, can be carried out in less than 45 minutes.[11] A fully integrated instrument that will provide sample in to results out forensic DNA analysis will dramatically reduce the costs (including labor, space and validation) of establishing and operating a forensic DNA lab.

Conclusion

These smart microfluidic platform will have a major impact on artificial intelligence and biological computations. The portability and ease-of-use microfluidic platforms certainly seems poised to broaden up the applications of DNA fingerprinting beyond forensic crime-solving such as for infectious disease detection or for other types of measurements based on molecular tests by just changing the cartridge and the assay chemistry and run the system. Effective DNA databases are being constructed and existing core loci have played and will continue to play a vital role in human identity testing.

Although there are many more details to work out to improve the efficiency of microfluidic systems, researchers hope that the time invested in validating microfluidic measurements will pay off in the long run. This technological revolution in forensic science could ultimately lead to a paradigm shift in which a new role of the forensic expert emerges as developer and custodian of integrated forensic platforms. Many more exciting scientific and technological advances are still on the horizon, there is no doubt that the future

landscape of forensic DNA analysis will look very different from what we see today.

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