Review Article

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Molecular Techniques used for Determination of Genetic Identity in Forensic Samples

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Abstract

Molecular techniques in forensics is gaining importance in medico-legal and criminalities identification after establishment of molecular methods such as DNA (Deoxyribose Nucleic Acid) finger printing to identify individuals. The principles and techniques used for forensic means; modern DNA profiling procedures that are widely used in resolving various issues of inheritance or succession, identifying missing child, paternity dispute, murder and sexual assault, adoption maintenance of minor child and victim of mass disasters. Nuclear DNA evidences have been recovered from blood, semen, saliva, skin and hair roots. DNA base techniques such as PCR (Polymerase chain reaction), RFLP (Restriction fragment length polymorphism), VNTR (variable number of tandem repeats), STR (Short tandem repeats), Y-chromosome analysis and Mitochondrial analysis are being used for investigation. DNA-based evidence has become a significant supporting tool for law enforcement agencies/investigators to solve difficult crime cases and give right decision and judgment.

Keywords: Molecular Forensic; DNA Profiling; PCR-RFLP; STR- Genotyping; NGS; Microarray.

Introduction

Molecular forensic is method of extracting evidence from biological samples collected from the crime scene. Over the last 30 years such biological samples have revolutionized the forensic investigation i.e. Analysis of DNA. All biological materials contain DNA and all DNA carries identity of each individual. Dr Alec Jeffreys introduced DNA finger printing technique first time to identify individuals in 1984 at University of Leicester [1,2].

DNA based techniques such as PCR (Polymerase chain reaction), RFLP (Restriction fragment length polymorphism) have been used for investigation to analyse VNTR (variable number of tandem repeats), STR (Short tandem repeats),Y-chromosome and Mitochondrial analysis from biological samples. The focus of most criminal investigations is on linking the evidence after discovery from the crime scene to suspects; so genetic science has played an important role in this process. Currently, forensic DNA profiling by PCR based STR markers have been found to be suitable for forensic application. A panel of multiple–allelic STR markers have been developed and standard protocol have been validated in the laboratories worldwide using capillary electrophoresis. The incorporation of these STRs into commercial kits have improved the application of DNA evidence with reproducible results from the less nucleated cells or even severely compromised materials. RFLP for VNTRs (long motifs) have been replaced due to labour intensive and statistical errors, chances of cross-contamination and inappropriate forensic samples. Furthermore, allelic profile can be generated from Mitochondrial DNA (recovered from both bone and teeth dating back to thousands of years) and applied in anthropological domain. DNA fingerprinting is one of the most significant advances in forensic science

in the era of today's criminology [3,4]. Now in the emergence of Next generation sequencing (NGS) more forensic data can be generated with faster analysis in future. In this article molecular methods applied in the analysis of genetic material (DNA) of forensic samples will be highlighted and discussed.

Development of DNA Fingerprinting

Sir Alec John Jeffreys, a British Geneticist born on 9th January 1950, first time developed techniques for DNA fingerprinting and DNA profiling in the field of forensic genetics in 1984. Dr. Jeffreys unexpectedly observed that DNA patterns of different people showed both similarities and differences while working in his laboratory at Leicester on samples of his technician's family. He realized that there are possibilities that DNA fingerprinting can be used for recognizing the variations in genetic code to identify individuals. He also found that some regions of DNA sequences were repeated again and again in a sample and he developed a technique to examine the length variation of these repeat sequences and created the test to perform human identity test. These repeated pattern of DNA are known as VNTRs (variable number of tandem repeats) and were detected by a technique called RFLP (Restriction fragment length polymorphism). In forensic genetics STRs (Short tandem repeats) known as microsatellites and minisatellites are generally performed to solve casework by using PCR (Polymerase Chain reaction) technique. Microsatellites and Minisatellites are together known as VNTRs [5,6]. Thus, DNA profiling based on typing of individuals, highly variable minisatellites in the human genome was also developed by Alec Jeffrey and his co-workers in 1985.

Biological Samples for Forensic DNA Profiling

All biological samples that possess nucleated cell can be considered for DNA profiling. The proper collection of samples from crime scene or for paternity testing and their proper storage and transportation to the laboratory for investigation is very important to minimize the contamination and degradation of DNA present in it. Ideally biological samples should be collected within 24 hours from the crime scene, after that degradation occurs as a result of enzymatic action of cells. To avoid the contamination, one should wear gloves all the time, avoid coughing and sneezing during the collection of samples. As a general rule samples of fluids should be refrigerated and anything else should be kept dry in paper envelopes. Samples should be clearly labelled with name, date and time of collection and transported safely in correct manner. The biological samples that are present at crime scene or on victim's body and for paternity disputes, such as blood and semen (liquid /dry deposited on support), biological secretions in case of sexual acts (saliva, semen, mixture of secretions), buccal swab, hard tissues (bone, teeth), hairs with follicles, skin cells, brain cells, muscles, tissue, in some cases fingernails should be collected. Eventually blood, saliva and semen are the main sources of DNA profiling in forensic genetic testing [7].

Isolation of DNA from Forensic Biological Samples

Forensic evidence when sent to laboratory for profiling, the genetic material (DNA) is extracted from the samples by organic method (phenol-chloroform method). Briefly to the sample an equal volume of forensic buffer should be added, incubated at -20°C for 2 hours and then incubated at 60°C for 10 minutes. The samples are washed with PBS, followed by centrifugation and then refrigerated. Now forensic buffer, 20% SDS and Proteinase K (20 mg/ml) are added to the washed sample and incubated for 16 hours at 37°C. After incubation, phenol extraction is performed to remove cellular debris by separating aqueous layer using centrifuge. The DNA is precipitated by adding 3 M Sodium Acetate and Absolute Alcohol, and then DNA is dried to dissolve it in T.E. buffer for further use [8,9].

Quantification of Isolated DNA

DNA quantification can be performed by various methods. The commonly used commercial method is measuring absorbance of DNA samples at 260 nm and 280nm on spectrophotometer. Spectrophotometry is based on the interaction of substance with incoming radiation.The DNA purity/quality can be computed from the ratio absorbance of DNA samples at 260 nm and 280nm, if absorbance quotient (Q) is 1.7 to 2 the DNA is considered to be pure. This technique is not species specific so if there is bacterial or fungal DNA contamination it can affect the profiling results. It is important one should maintain sterility during DNA extraction and quantification to avoid contamination [10].

VNTRs Analysis based on RFLP

In this technique high molecular weight targeted DNA is digested with restriction enzyme which has recognition sites at both ends of hyper variable regions. The fragmented DNA after digestion are

arranged in specific order by the number of repeats of sequence, these fragments are separated (according to size) by agarose gel or by polyacrylamide gel electrophoresis and detected by using labelled VNTR probes. A unique pattern of DNA fragments of multiple VNTR loci on gel is compared with known and unknown origin of DNA samples and analysed on the basis of similarities and dissimilarities of patterns. RFLP analysis of VNTR loci is a good method to resolve the paternity disputes where DNA can be extracted from blood samples. However, this technique is not performed on other forensic investigations because high molecular weight DNA are required. Therefore, analysis of VNTR is limited to investigation where large amount of DNA is recovered. This method has been replaced due to statistical errors and chances of cross contamination and now PCR based STR profiling is done worldwide for forensic investigation [11,12].

STR Analysis based on PCR

Short tandem repeats (STR) loci are a class of polymorphic markers that are present throughout the human genome and consists of tandemly repeated sequence (1 - 6 base pairs) in length [13]. Their abundance, hyper-variability and amenability to amplification by Polymerase chain reaction (PCR) makes them ideal markers for use in the identification of individuals. PCR is an in-vitro method for amplifying specific DNA sequence. Starting with trace amounts of a particular nucleic acid sequence of any source, PCR enzymatically generates millions of exact copies, thereby making genetic analysis of tiny samples a relatively simple process. PCR was invented by Kary & Mullis of Cetus Corporation in 1983 and is widely used technique in molecular biology with direct application in genetic research, medical diagnostics, forensic science & paternity testing. This process requires pairs of primers to amplify target DNA sequence that hybridize (Stick) to the target DNA (3 prime end to 5 prime end), four dNTPs (deoxyribonucleate triphosphates), heat stable taq DNA polymerase and DNA template (extracted DNA). The PCR process consisted of three step cycles: denaturing step, annealing step and extension step.

1. *Denaturing step*: In this process double stranded targeted DNA molecules are denatured (separated by heating) into single strands by incubating them at high temperature (usually 94°C for 60 seconds).
2. *Annealing step*: In this step temperature is lowered down to allow the primers to specifically bind (anneal) to their complementary target (flanking) sequence. This is usually done at 45°C-65°C depending upon GC content of primers.
3. *Extension reaction*: In this step usually, temperature is raised to 72°C during which the annealed primers are extended on DNA template by a thermostable DNA Taq Polymerase to allow the synthesis of DNA region.

The above steps comprise as one cycle (denaturing DNA to synthesis). This process is repeated for about 30-40 cycle on thermocycler (PCR machine). In this process the completion of each cycle doubles the number of DNA molecules [14].

The DNA regions with repeat units which is 2-6 base pairs in length are STRs, polymorphic STR loci can be copied simultaneously by multiplex PCR. Currently, in forensic investigation and paternity testing multiplex STR typing or profiling are being used to study 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA) and one gender marker Amelogenin by Capillary Electrophoresis (CE) on Genetic Analyzer [15].

PCR Amplification for 15 STR Loci

PCR is performed by using commercially developed STR multiplex kit for autosomal and Y-chromosome markers. It amplifies specific product at the X and Y- chromosomes which are 107 bp and 113 bp long (each pair of primers fluorescently labelled to distinguish profiles). The characteristics of 15 loci are shown in Table 1. For PCR amplification of STRs; in a PCR tube 10 µl of Master mix is transferred, which contains reaction buffer and taq polymerase, 5 µl set of primers and extracted DNA template (1-5 ng) as recommended by manufacturer. After mixing the reaction is run in thermocycler machine with the amplification condition such as initial denaturation at 95°C for 11min; 28 cycle of 94°C for 1min (denaturation), 59°C for 1min (annealing), 72°C for 1min and final extension of 60°C for 60 min. The PCR product is taken out and kept in the deep freezer (-20°C) till it can be used for genotyping in Genetic analyser [16,17,18].

STR Profiling (Genotyping)

To analyse the profile the PCR product (1 µl) is mixed with commercially available Hidi Formamide (8.7 µl) and GeneScan 500 LIZ (0.3 µl). it is denatured at 95°C and chilled at deep freezer. The sample is loaded into each well containing PCR product along with one standard and one negative control in micro-well plate and run in the Analyzer using Polymer

Table 1:

16 Loci	Locus (Genetic Markers)	Chromosome location	Repeat motifs	Allelic range	Primer labelled
1	D8S1179,	8q	TCTA	8-19	6-FAM
2	D21S11	21q11-21	TCTA	24-38	6-FAM
3	D7S820	7q11.21-22	GATA	6-15	6-FAM
4	CSF1PO	5q33.3-34	AGAT	6-15	6-FAM
5	D3S1358	3p	TCTA	12-19	VIC
6	TH01	11p15.5	AATG	4-13.3	VIC
7	D13S317	13q22-31	TATC	8-15	VIC
8	D16S539	16q24-qter	GATA	5-15	VIC
9	D2S1338	2q35-37.1	TGCC	15-28	VIC
10	D19S433	19q12-13.1	AAGG	9-17.2	NED
11	vWA	12p12-pter	TCTA	11-24	NED
12	TPOX	12p23-pter	AATG	6-13	NED
13	D18S51	18q21.3	AGAA	7-27	NED
14	D5S818	5q23.3-32	AGAT	7-16	PET
15	FGA	4q28	TTTC	17-51.2	PET
16	Amelogenin	X: p22.1-22.3, Y: p11.2	Gender markers	X, Y	PET

4 with capillary to generate the profiles. Fragments are separated according to their length by electrophoresis. The data is analysed with software to assess the quality of amplification and fragment length of each STR marker that is recorded as series of number (number of repeats) assigned to specific alleles at every chromosomal locus [19].

Analysis for DNA profiling in Crime/ Casework/Paternity Disputes

The DNA profiling generated as fragment length or STRs by genetic analyser has to be compared with the Profile of suspect with the DNA profile of evidence. The samples collected from suspects including victim and other person present at the time of crime are referred as Reference Samples. If DNA profiles of reference and evidence are found to be identical, then it is considered as inclusion (match); if they are not identical it is considered as exclusion. In terms of Inclusion it is important that the pair of profiles are perfectly similar at each and every locus with the profile of suspect and profile of evidence. If pair of profiles are different or does not match in certain locus, it should be reported as partial match or exclusion. Similarly, in Paternity case matching of DNA profiles of child, mother and disputed fathers, the child (either son or daughter) will inherit half of their autosomal alleles from each parent and the son will inherit a Y-chromosomal allele from the father and daughter will inherit X-chromosomal alleles from the father. The male child will match the biological father at all Y- chromosome loci, whereas the female child will match the biological father at all X-chromosome loci. Statistics will help to determine if an alleged father should be included or excluded as the father of the child, because no known phenotype are associated with STR loci, so only genotype of parents and child should be considered [20,21,22].

Furthermore, the statistical calculation is used in forensic science to assess the strength of evidence and the probability that there could be random match to some other person. In paternity cases, likelihood ratios and combined probability of exclusion are two calculations that can be performed to evaluate the certainty of the evidence [23,24]. Therefore, DNA analysis of any physical evidence is a very strong form of technology which is exceptionally accurate – DNA evidence doesn't lie!

Automation in STR Analysis

Human identity testing has been widespread by using DNA typing methods. During the last decade tremendous growth in the use of DNA evidence in crime scene investigations, paternity testing, unidentified body and missing persons have been reported.

As demonstrated above many molecular techniques have been used earlier and even currently, depending on PCR based capillary electrophoresis (CE) on Genetic Analyzer. Now various commercial kits with semi-automated and automated DNA extraction are available to generate STR profiles associated with biological evidence to avoid cross contamination. However, the procedure is still labour intensive and time consuming in handling the biological samples of decomposed unidentified body. Such constraints for the STR typing limit expeditious processing of results. To provide expeditious STR

profiling numerous Rapid DNA instruments have been developed to perform DNA extraction, PCR, STR profiling and interpretative result in less time. STR profiling by using 24 loci, 10 mini STR loci and global filer express kit with allelic ladder are available [25,26].

Next Generation Sequencing for Forensic DNA Analysis

The next generation sequencing is the high throughput sequencing technique for DNA and RNA. This technique is more quick and cost-effective than Sanger sequencing and has revolutionized the study of genomics and molecular biology. STR analysis is likely to remain the most important and commonly used genetic technique in forensic science for the foreseeable future. When NGS technology was firstly introduced to genomics, it was not suitable for STR testing because the read length was generally too short. With technological advances, the average read length has been continually increasing. Since alleles with similar length can be easily distinguished using NGS technology and digital read count could significantly facilitate the identification of mixed DNA samples and analysis of complex paternity cases, some researchers have recently started using NGS technology for STR testing. To process the forensic NGS data, various workers developed software such as STRait Razor, software that can analyse the NGS data for 44 STRs, including 23 autosomal and 21Y chromosome STRs. Illumina's MiSeq system establishes reference allele database to detect single source and mixed DNA samples and observed that most locus genotyping results were stable and reliable [27]. NGS technologies are going to be crucial for human DNA genotyping in cases like mass disasters or other events where forensic specimens and samples are compromised and degraded. With the use of NGS it will be possible to achieve the simultaneous analysis of the standard autosomal DNA (STRs and SNPs), mitochondrial DNA, and X and Y chromosomal markers.

NGS technology has many potential advantages for STR analysis. These include high throughput, low cost, simultaneous detection of large numbers of STR loci on both autosomes and sex chromosomes and also the ability to distinguish alleles with similar length or digital read count. NGS technology would therefore significantly facilitate the identification of mixed DNA samples and analysis of complex paternity cases, and ultimately greatly increase the efficiency and cost-effectiveness of legal cases [28,29].

Application of Microarray in Forensic

DNA microarrays have provided to be a new and powerful tool to perform important molecular biology and clinical diagnostic assays. "Microarray" refers to a microchip-based testing platform that allows high-volume, automated analysis of many pieces of DNA at once. A DNA microarray is an ordered arrangement of oligonucleotides attached to a solid support used to analyse nucleic acid samples via hybridization.

The term "DNA microarrays" was first used in an assay that examined the expression of multiple genes in parallel. The development of this technique was originally derived from Southern Blotting, developed by Professor Ed Southern of the University of Oxford in which fragments of DNA were relocated from an agarose gel to a cellulose nitrate filter where they were hybridized to radioactive RNA probes. Following the hybridization, autoradiography was employed to confirm the presence of the labelled DNA, based on the sequences of the RNA probes. From this protocol the field of DNA microarrays has exponentially grown due to the fact that it can be implemented in a large number of scientific fields. Beginning with Southern's methodology, the use of DNA microarrays has become a standard tool for molecular biology research and clinical diagnostics. DNA microarrays have generally been used to detect bacterial pathogens commonly found in water and food, immunological assays, PCR-based assays, electrochemical assays and array-based biosensors.

The field of molecular forensic has implemented DNA microarrays for SNP genotyping. Analysis of SNPs within the entire genome, by investigating short sequences of DNA that include a single base pair change, can be used to create a unique profile of SNPs for an individual. The use of SNPs in forensic is common because they occur frequently, with estimates of 1 for every 1,000 base pairs and over three million are present in the genome. An array-based format not only can test thousands of SNPs at once but is highly successful when handling degraded or minute amounts of DNA such as in crime scene investigations [30]. The field of forensics often must solve problems requiring parental identification. Individual identification using microarray platforms designed to genotype SNPs provide a viable method of such testing. The use of DNA microarrays as an efficient assay to sequence a genome through multiple hybridization experiments in parallel has been previously reported. For Chromosomal Micro Array (CMA) a chip uses labels or probes that bond

to specific chromosome regions. Computer analysis is used to compare a patient's genetic material to that of a reference sample. The basic idea behind DNA microarray technology has been to immobilize known DNA sequences referred to as probes in micrometre-sized spots on a solid surface (microarray) and specifically hybridize a complementary sequence of the analyte DNA or a target.

A fluorescent labelled reporter facilitates fluorescence detection of the presence or absence of a particular target or gene in the sample. By using laser scanning and fluorescence detection devices such as CCD cameras, different target hybridization patterns can be read on the microarray and the results quantitatively analysed [31].

Conclusion

In the era of new generation technologies, in our opinion the most popular methods are those that are commercially available in an easy to perform either in the kit format or not technically demanding. Improved next generation sequencing (NGS) and DNA Microarray are all being applied for DNA profiling for forensic purposes in future. It is inevitable that these techniques will become the standard technology globally for DNA base profiling. With passage of time, more developed techniques are being used, specifically suited for various investigative purposes such as methylation- specific PCR.

DNA profiling is a tool that is not only used to apprehend the guilty but also to exonerate the innocent. DNA evidence thus unravels the truth-it never lies. The Passage of time does not affect it and neither does it change. Thus, the use of DNA profiling and Molecular Forensics have opened a new era in Forensic investigations. It is important to remember that DNA evidence is not the only form of evidence in a case and that other supporting evidence will still be needed by a court of law to convict a person of a crime.

Conflict of Interest: None

Reference

1. Jobling MA, Gill P. Encoded evidence: DNA in Forensic Analysis. Nature. Rev. Genetics. 2004;4:739-51.
2. Piotrowski P, Grzybowski T, Liwka K. The possibilities of applying molecular biology techniques to forensic toxicology. Problems of Forensic Sciences, 2003;LIII:7-21.
3. Budowle B and Van Daal A. Extracting evidence from forensic DNA analsyes: Future molecular biology directions. BioTechniques 2009;46:339-50.
4. Rodrigues E.J. Banaulikar S. Forensic DNA applications and Forwarding of Biological Materials. J Forensic Med &Toxi; 2007;24(2):61-65.
5. Roewer L. DNA fingerprinting in forensics: past, present, future. Roewer Investigative Genetics, 2013; 4:22.
6. Shrivastava P, Trivedi VB, Singh AK, Mishra N. Application of DNA Fingerprinting Technology in Forensic Investigation. Int. J Scientific Res. Publication. 2012;2:1-4.
7. Butler JM. Sample collection, DNA extraction and DNA quantitation Fundamentals of Forensic DNA Typing. Academic press, imprint of Elsevier. 2009; 33-44.
8. RapleyR. Basic Molecular Biology Techniques School of Life Sciences, University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK Molecular Biology and Biotechnology, 5th Edition Edited by John M Walker and Ralph Rapley Royal Society of Chemistry 2009.Published by the Royal Society of Chemistrywww.rsc.org.
9. Chacon-Cortes D and Griffiths LR. Methods for extracting genomic DNA from whole blood samples: current perspectives. Journal of Biorepository Science for Applied Medicine. 2014;2:1–9.
10. ElkinsKM. Forensic DNA biology, Laboratory Manual. Determination of DNA Quality and Quantity using UV-Vis Spectroscopy. Academic Press is an Imprint of Elsevier. 59-62.
11. Rimoin DL, Connor JM, Pyeritz RE, and B.R. Korf (Eds.), Emery and Rimoin's Principles and Practice of Medical Genetics, 5th ed. Vol. 1. Elsevier, Philadelphia.
12. Jeffreys AJ, WilsonV, and Thein SL. Hypervariable minisatellites regions in human DNA. Nature. 1985; 314:67-73.
13. Ruitberg CM, Reeder DJ, Butler JM. STR Base: a short tandem repeat DNA database for the human identity testing community. Nuclei Acid Researc. 2001;29: 320-22.
14. Kavya SR. PCR Technique with its Application. RRJMB. 2015;4:1-12.
15. Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM and Budowle B. Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. J. Forensic Sci. 2001;46:647-60.
16. Butler JM andHill CR. Biology and genetics of new Autosomal STR loci useful for Forensic DNA Analysis. Forensic Science Review 2012;24:15-26.
17. Christian M. Ruitberg, Dennis J. Reeder and Jhon M. Butler: STR Base: a short tandem repeats DNA

database for the human identity testing community. Nucleic Acids Research.: 2001;29(1):320-22:

18. Krenke, BE, TerebaA, AndersonSJ, BuelE, CulhaneS, FinisCJ, et al. Validation of a 16-locus fluorescent multiplex system. J. Forensic Sci.200; 247:773-85.
19. Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. Forensic Sci. Int. 2000;112:17-40.
20. Hanson EK, Ballantyne J. Whole genome amplification strategy for forensic genetic analysis using single or few cell equivalents of genomic DNA. Anal Biochem. 2005;346:246–57.
21. Butler JM, STR Genotyping and Data Interpretation. Fundamentals of Forensic DNA Typing. Academic press, imprint of Elsevier. 2009.p.205-228.
22. Bieber FR., BrennerCH, and LazerD. Human genetics. Finding criminals through DNA of their relatives. Science 2006;312:1315-16.
23. Yamamoto T, Tamaki K, Huang XL et al. The application of minisatellites variant repeat mapping by PCR (MVRPCR) in a paternity case showing false exclusion due to STR mutation. J Forensic Sci; 2001; 46:374-8.
24. Pant PV, Tao H, Beiharz EJ, Ballinger DG, Cox DR, Frazer KA. Analysis of allelic differential expression in human white blood cells. Genome Res; 2006;16:331-9.
25. Wang DY,Gopinath S,Lagace RE,Norona W, Hennessy LK, Short ML,MuleroJJ. Developmental validation of Global Filerexpress PCR amplification Kit:A 6-dye multiplex assay for the direct amplification of reference samples. Forensic Science International: Genetics. 2015;19:148-155.
26. Thong Z, Phua YH, Loo ED, Shue BH,SynCKC. Investigative leads from DNA Casework experience from the Integen X RapidHIT™200 System. Forensic Science International: Genetics supplement series 5:e69-e70:015.
27. Bornman D.M, Hester M.E, SchuetterJ.M, Kasoji M.D, Minard-Smith A., Barden C.A. et al. Short-read, high-throughput sequencing technology for STR genotyping. Biotechniques 2012.pp.1-6.
28. D.H. Warshauer, D. Lin, K. Hari, R. Jain, C. Davis, B. LaRue, et al. STRait Razor: a length-based forensic STR allele-calling tool for use with second generation sequencing data. Forensic Sci Int Genet, 2013;7:409-417.
29. Irwin J, Just R,ScheibleM,LoreilleO, Assessing the potential of next generation sequencing technologies for missing persons identification efforts.Forensic Sci Int Genet Suppl Ser 2011;3:447-8.
30. Lindroos K, Sigurdsson S, Johansson K, Ronnblom L, Syvanen AC. Multiplex SNP genotyping in pooled DNA samples by a four-colour microarray system. Nucleic Acids Res; 2002;30:70.
31. Brewster JL, Beason KB, Eckdahl TT, Evans IM. The microarray revolution: Perspectives from educators. BiochemMolBiol Edu; 2004;32:217-27.