

Forensic Identification by Using Insertion-deletion Polymorphisms

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ABSTRACT Forensic science is basically the determination of the human identity. With advances in science and technology and the availability of DNA profiling, it is possible now to determine the identity even in decomposed and mutilated bodies and even with parts of a body as trivial as a hair or a drop of a blood. Methods and materials: Short tandem repeat polymorphism (STRP) genotyping and single nucleotide polymorphism (SNP) are the well-known DNA profiling methods used in the forensic field, but having drawbacks like need of big amplicons, having higher mutation rates and requiring complicated procedures, and being expensive respectively. Insertion Deletion polymorphism (INDEL) is a natural genetic variation due to the insertion or deletion of nucleotide in the human genome, and INDEL typing requires smaller DNA samples and are the most abundant variations in the human genome. INDELS are analysed with a simple fluorescent PCT followed by capillary electrophoresis, at present Qiagen Investigator DIPplex® kit of 30 INDELS and a 38-INDEL multiplex assays are available. Objectives: INDEL typing is useful in forensic genetics, population genetic studies, and medical genetics as in human and kinship analysis. With the advent of INDEL-STP typing technique an autosomal DNA profile of a minor donor is also plausible. Conclusion: Personalized therapy is possible with INDEL typing as it is helpful in determining the source of genetic diseases. This paper explores the application of INDELS in forensic identification.

INTRODUCTION

Forensic science is the application of science to legal problems; that is, science applied to criminal cases, national security, as well as civil and administrative matters (Bush 2015). Mostly, forensic science works on criminal cases, such as homicides and sexual assaults; yet, forensic science is also applied to civil matters, for example, disputed paternity and product failures (Dobash and Dobash 2015). In term of criminal case, forensic science is applied to investigate the corpus delicti, to identify substances and individuals, to provide investigative clues, to develop linkages in the case as well as to support or disprove statements by witnesses, victims or suspects (Sefanyetso 2009). In fact, forensic identification or human identification can be indeed challenging in most cases. Basically, by using old methods, an individual can be identified by his morphological characteristics, fingerprint, teeth, body tissues, body fluid,

bone, tattoo or body piercing (Vij 2011; Shepherd 2003). Unfortunately, these old methods display drawbacks and are inapt in the identification of victims of fire, explosion, air crash, or road accidents, when the body is incomplete, highly decomposed or skeletalised. Over decades, forensic scientists have been working diligently to invent new methods to overcome the obstacles in forensic identifications. As technology advanced, scientists have eventually invented DNA profiling as the latest strategy in forensic identification. Under DNA profiling, there are two common methods, namely short tandem repeat polymorphism (STRP) genotyping and single nucleotide polymorphism (SNP) genotyping, which are usually used for identification. STRs or short tandem repeats are tandemly repeated DNA segments with repeat lengths up to 6 bp and total lengths commonly less than 60 bp, in human genome (Hameed et al. 2014). On the other hand, SNPs or single nucleotide polymorphisms are the most abundant form of genetic variation in humans (Twyman 2013). Both STRPs and SNPs show natural variation in a population, and thus, can be genotyped and analysed for forensic identification. At present, STRP genotyping is known as the standard procedure in forensic identification, however, STRPs have high mutation rates and big amplicons are needed in STRP analysis, which makes

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it inapplicable in deficiency cases (Schneider 2012). As an alternate method, SNP genotyping has covered the limitation of STRP genotyping method, as SNPs have low mutation rates and can be analysed with small amplicons (Pereira et al. 2012). Even so, SNP typing technique has its own limitation, for instance, complicated procedures and expensive, advanced technologies are all needed to genotype SNPs in human genome (Twyman 2013). With no doubt, the methods and technologies used for forensic identification are far from advances, making the identification even more challenging. To overcome these obstacles a new technique called the insertion deletion polymorphism (INDEL) typing which was discovered recently, can aid in identification even when very small amount of DNA sample is available in the crime scene. Furthermore, INDEL is one of the most abundant genetic variations in human genome, which is easier to be genotyped compared to STRP and SNP (Reid 2013).

Besides its application on forensic genetics, INDEL typing is as well applicable in population studies and medical genetics (Børsting and Morling 2015). In this review, the methodology for the detection of INDELs, its applications as well as its advantages and disadvantages are highlighted.

Literature Review

Introduction of INDELs

Over the decade, several types of genetic variations have been discovered and studied for forensic genetics. INDEL is one of the natural genetic variations, ranging from 1 to 10,000 bp in length, which is resulted from insertions or deletions of one or more nucleotide in human genome (Pereira et al. 2012). In fact, INDELs have received much less attention than other forms of variations, despite INDELs are abundant in human genome (Mullaney et al. 2010). But, how abundant is it? Back in 2002, a research conducted by Weber had identified and characterised 2,000 diallelic INDELs distributed throughout the human genome, and those INDELs had been proven highly abundant, could be easily analysed and applicable in many different fields (Weber et al. 2002; Mullaney et al. 2010). Ever since then, many researches were conducted and further proved that INDELs are the second most abundant variation after SNPs and cause an

apparent genetic variation in human genes (Mullaney et al. 2010). In addition, in 2006, a research reported the five major classes of INDELs, including (i) insertion and deletions of single-base pairs, (ii) monomeric base pair expansions, (iii) multi-base pair expansions, (iv) transposon insertions, and (v) INDELs containing random DNA sequences (Mills 2006). In 2011, studies revealed that some INDELs are located in the promoters and axons of genes, which are also known as coding INDELs (Mullaney et al. 2010). Coding INDELs are likely to influence gene function, as well as human traits and health (Mills 2006). For example, the well-known genetic disease, cystic fibrosis, is commonly caused by a coding INDEL within the CFTR gene (Mills 2006). Therefore, INDELs are considered one of the most influential polymorphisms in human genomes.

OBSERVATION AND DISCUSSION

INDEL typing is well-known for its simplicity. In other words, INDELs can be easily analysed with a simple fluorescent PCR followed by capillary electrophoresis (Fondevila et al. 2012). At present, there are two multiplexed INDEL typing assays available for forensic identification, namely Qiagen Investigator DIPplex® kit of 30 INDELs and a 38-INDEL multiplex assay (LaRue et al. 2012). In contrast to SNP typing which is best done by complex microarray hybridisation, the procedures for INDEL typing are simple and time saving (Pena et al. 2012). Generally, INDEL typing procedures include selection of marker, design of specific primer, amplification and analysis of INDELs (Pereira et al. 2012). Mostly, markers used in a research are chosen from the NCBI dbSNP, based on the following criteria: (i) non-coding and diallelic, (ii) allele length variations of 2 to 6 bp (Oka K et al. 2013). Next, the primers are usually designed by using Primer 3 software to create amplicons with different size range from 50 to 160 bp in length (Castella et al. 2013). In Qiagen Investigator DIPplex® kit, PCR amplification is performed with 30 PCR cycles, meanwhile, in 38-INDEL multiplex assay, PCR amplification is performed with 29 PCR cycles in two stages (Fondevila et al. 2012; Wang et al. 2014). The PCR products from both assays are then diluted and run on 3130 Genetic Analyser using POP4 as separation polymer, 36 cm capillary array, and appropriate dye, to detect INDEL markers in the sample (Fondevila et al. 2012).

Applications of INDEL Genotyping Technique

In the past five years, the application of INDEL typing is escalating in many different realms, such as forensic genetics, population genetic studies, medical genetics. In forensic genetics, INDEL typing technique is applied to human identification as well as human and kinship analysis. Just two years back, a research conducted by Pena has proved that an assay of 40 INDELS has excellent potential for forensic identification (Pena et al. 2012). In details, the research was done by using a simple multiplex system for the typing of 40 carefully chosen INDELS in polyacrylamide gels with silver staining, which can be used as the alternatives for more complex fluorescent typing (Pena et al. 2012). As most criminal cases produce unbalanced mixed DNA samples, with large amount of victim's DNA and trace amount of perpetrator's DNA, the identification of the perpetrator by using STRP typing method alone is not possible (Hall et al. 2011). Therefore, researchers have invented a new method, called INDEL-STP typing technique, which is formed by combining INDEL typing to STRP typing (Hall et al. 2011). Recent study has proven the ability of this method to produce high resolution autosomal DNA profile of a minor donor who contributes less than 0.1 percent to a DNA mixture (Hall et al. 2011). In addition, a research conducted in 2011 has stated that human identification of body for tumour tissue might be necessary in some forensic circumstances (Zhao et al. 2011). The same research has pioneered the application of INDELS in human gastrointestinal tumour tissues for identification purpose (Zhao et al. 2011). Besides, INDEL typing also plays an important role in paternity and kinship analysis. As alternative to currently applied STRP typing in paternity and kinship analysis, INDELS have low mutation rates and can be typed more easily on instruments available in all forensic laboratories, making the analysis more reliable and easier to be performed (Pimenta et al. 2010). Another interesting application of INDEL typing is in population genetics studies. In 2009, a successful study had proven INDEL typing as an effective method for estimating individual and global ancestry proportions in admixed populations (Santos et al. 2010). Apart from that, INDEL typing can also be applied to medical genetics, to predict the future health of an individual, to

determine the source of genetic diseases as well as to provide personalised medicine for the individual (Mullaney et al. 2010).

Advantages of INDEL Genotyping Technique

Besides the benefit of being very useful in many different applications, there are many more reasons showing why the INDEL typing is preferred over the other variations in forensic genetics. In most criminal cases, the lack of DNA sample in the crime scene has always been the biggest challenge to forensic identification and it is almost impossible to solve a criminal case without firm, accurate human identifications. In the past ten years, STR typing has been developed as the standard DNA strategy applied in forensic genetics due to its easy interpretation, however, STR typing is not perfect in the sense of its inability to type highly degraded samples and high mutation rates (Da Costa Francez et al. 2012). Later, the discovery of SNP typing has overcome the obstacle when this typing is able to type highly degraded DNA samples, by using complicated genotyping protocol and expensive technologies (Pereira et al. 2009). As an alternate strategy, INDEL typing combines all the desirable features of STR and SNP typing (Pereira et al. 2009). Studies showed that INDEL typing is very practical in forensic genetics because, (i) INDELS are in great abundance and distributed throughout the human genome, (ii) they derive from a single mutational activity, (iii) they have low mutation rate, (iv) no stutter, (v) short amplicon size (vi) INDELS can be genotyped by using simplified approach, (vii) INDEL typing can be done by using the same methods and technologies that are similar to that for STR typing, which are readily installed in a forensic laboratories (viii) the amplification of INDELS is possible in low amount degraded DNA samples (LaRue et al. 2012; Da Costa Francez et al. 2012). As a conclusion, INDEL typing is considered as a powerful, sensitive and cost-effective approach for forensic identification (Pereira et al. 2012; LaRue et al. 2012; Oka et al. 2013).

Disadvantages of INDEL Genotyping Technique

Apart from all the advantages discussed above, INDEL typing has its limitations. Research by Weber, stated that not all INDELS are highly informative in all populations, instead,

some INDELs have low informativeness in some populations (Weber et al. 2002). The same research had also proven that INDELs will be extremely low in species with more genetic diversity than humans (Weber et al. 2002). Meanwhile, other studies have also showed deficiency in the discovery of small INDELs, despite the fact that several million of INDELs are identified, the exact number of INDELs in human genome remains unknown (Mullaney et al. 2010). Moreover, when using INDEL typing for forensic identification, contamination in the DNA sample may be difficult to detect and could possibly lead to a false interpretation (LaRue et al. 2012).

CONCLUSION

The application of INDEL typing in forensic genetics should be emphasized and recommended highly due to its many applications in various realms: (i) INDEL typing can be done on different forensic samples as well as highly degraded DNA and highly decomposed or skeletalised decesses, which is impossible by using STR typing (ii) INDEL typing is proved to be useful in population studies as well as the studies of genetic diseases. (iii) Many studies have proved that INDEL typing can be applied to medical forensics, by which, genetic studies can be studied to an extended level.

RECOMMENDATIONS

INDEL typing may replace the application of STR and SNP typing for human identification, as well as may be used to predict human traits and future health. INDEL typing is an effective, powerful, valuable, and is a simple approach for forensic identification.

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