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Generating DNA profile from low copy number DNA: Strategies and associated risks

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ABSTRACT. Many shreds of evidence found on the crime scenes contain a trace amount of DNA which results in insignificant profiling results for subsequent comparison. This can nullify the potential evidence material and hamper investigation process. Over the years, different strategies have been employed by various DNA testing laboratories to create interpretable DNA profiles generated from low template of DNA. This review highlights different strategies used by forensic laboratories worldwide for creating complete DNA profiles from low copy number template for comparison purposes along with its associated risks for forensic purposes.

Keywords: STR profiling; forensic genetics; likelihood ratio; random match probability.

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Introduction

Since its arrival, forensic science has been helping mankind in solving various criminal and non-criminal cases. Evidence material collected from the crime scene is analyzed using the latest techniques in forensics to narrow down the investigation and to individualize a person. One of the most important evidence which has been widely accepted in the courts and found to individualize a person is DNA evidence (Lynch, 2003). Sometimes DNA based evidence in criminal and non-criminal cases is so small in quantity that it cannot be analyzed using standard laboratory procedures, so advanced techniques and methodologies are required for their analysis and ultimate acceptability in courts.

DNA STR (Short Tandem Repeat) profiling is an important technique for generating DNA profile that has revolutionized the forensic world. It makes use of the PCR reaction to simultaneously amplify several STR loci present in the genome. DNA evidence in forensics is used for comparison purposes while solving criminal and non-criminal cases like rape, sodomy, murder, dead body identification, burglary, paternity and proof-of-death (Luftig & Richey, 2001). Multiplex PCR technique has an outreaching effect for creating a DNA profile by amplifying multiple genetic loci in a relatively short time (Hill et al., 2007). It has been estimated that an easily interpretable DNA profile can be created from 0.2-2 ng of starting DNA amount with normal cycling conditions of 28-30 cycles of PCR. However, if the amount of DNA is less than 100 pg then it gets really difficult to get full DNA profile utilizing normal PCR conditions. The quantity of starting material of DNA is an important factor for successfully creating a DNA profile (Gill, Whitaker, Flaxman, Brown, & Buckleton, 2000; Petricevic et al., 2010). Low copy number (LCN) is the profiling technique which has been widely used all over the world for creating interpretable DNA profile. Many forensic shreds of evidence, such as touch DNA, yield so lesser amount of staring DNA that it becomes impossible to create a complete DNA profile using standard conditions of PCR. Forensic laboratories face difficulties in solving cases because potential evidence becomes incapable of providing necessary information regarding the case (Balding & Buckleton, 2009; Van Oorschot & Jones, 1997).

This review describes DNA profiling issues inherent in a low quantity of DNA evidence-and improvised strategies and modifications adopted by various forensic laboratories for successfully creating a DNA STR profile from low template DNA, and also describes risks and hesitant acceptability of DNA STR profiling results in courts associated with these strategies.

Methodology used for creating DNA profile from LCN

With the passage of time and advent of novel challenges, different methodologies have been employed for creating an interpretable DNA profile from LCN DNA.

PCR based methods for improved LCN technique

One of the methods is the increasing in the number of cycles during the polymerase chain reaction (PCR), and it is the most easy and commonly used method for tackling LCN As the number of cycles can be increased from 28 to 35 cycles but not more than 35 because this could cause the denaturation of Taq enzyme and incomplete amplification producing artifacts such as stutters and –A's (Bessekri, Aggoune, Lazreg, Bucht, & Fuller, 2013; Forster, Thomson, & Kutranov, 2008; Kloosterman & Kersbergen, 2003).This method proceeded first time at Forensic Science Service (FSS) in United Kingdom. In FSS, almost twenty-one thousand samples were used. In that test, the number of cycles was increased up to thirty-four, which increased the number of copies to almost more than three billion. The study compared the results obtained from the increased number of PCR cycles with the standard number of PCR cycles. It concluded that increasing cycles may increase efficiency (Gilbert, 2010; Gill et al., 2000; Rameckers, Hummel, & Herrmann, 1997).

Other method is the designing of primers to amplify different regions simultaneously. In most of the forensic laboratories, in addition to old and original primers, mini STR based primers are used. As these primers are thought to work efficiently due to production of small sized amplicons, designing primers against these mini STR sequences can provide a basis for amplification of DNA with the small quantity of starting DNA material. As these kits contain functionally efficient primers that can bind to small fragment of DNA, it has been proposed that these can be good candidates for amplification of LCN obtained from various forensic evidence (Butler, Shen, & McCord, 2003; Mulero et al., 2008). With the use of modified nucleotides in place of standard nucleotides, increase in the efficiency of polymerase chain reaction and proper functioning of these primers has proven effective in amplifying low copy number of DNA samples (Ambers et al., 2016; Strom & Rechitsky, 1998).

The nested PCR has been found helpful where blood and burnt tissues are the source for DNA. These kind of forensic evidences are commonly used in reverse fatherhood analysis and have been proved successful in solving many hideous criminal and parentage cases (Strom & Rechitsky, 1998). In addition, biogenetic markers, which identify methylation patterns, are being used successful for forensic biological fluid identification. Use of bisulfide modification and pyrosequencing using nested primers has proved effective in amplifying low quantity of starting DNA material (Madi, Balamurugan, Bombardi, Duncan, & McCord, 2012).

Most of the forensic DNA kits recommend the starter quantity of 0.5 ng-2.0 ng template DNA for generating complete and reliable DNA STR profile. As of late, Whole Genome Amplification (WGA) techniques have been proposed as pre-amplification devices. It helps in amplification of the entire DNA template rather than selected genetic loci leading to several nanogram of DNA with starting quantity of picogram. This amplified input quantity can fulfill the optimal requirement of different forensic STR typing kits and can give accurate and interpretable DNA profiling results. The use of WGA and multiple displacement amplification has been proven beneficial for generating STR profile from LCN and newer methodology arising in the field of WGA can prove useful for generating various STR's and SNP's DNA profiles (Balogh et al., 2006; Maciejewska, Jakubowska, & Pawłowski, 2013; Schneider et al., 2004)

As PCR is used for making copies of a template DNA, therefore, by reducing the volume of PCR reaction mixture, the quality of the DNA template will not change. It has been found that it not only increases the sensitivity of the reaction by utilizing low quantity of sample, but also proves to be relatively inexpensive. The use of reduced volume of PCR reaction mixture requires fewer efforts and materials to detect the PCR product. There have been various modifications of this phenomenon. In some cases, DNA template is reduced in proportion with the entire reaction volume, while in other cases, template DNA quantity remains the same but reaction volume is halved. The reduction in volume has been found closely related with more fluorescence signals of STR's obtained in genotyping results. In other words, it concentrates the PCR product and hence more peak heights of STR alleles are obtained from the low quantity of template DNA (Bessekri et al., 2013; Gaines, Wojtkiewicz, Valentine, & Brown, 2002).

PCR reaction mixture contains all the ingredients necessary for a successful amplification process. By changing or modifying any of the basic components of PCR, the reaction efficiency can be improved. To

improve the sensitivity of the PCR reaction, concentrations of MgCl2 and Bovine Serum Albumin (BSA) have been proved efficient in increasing the sensitivity of PCR reaction. It prevents inhibition causing substances, such as phenol, from binding to Taq polymerase which ultimately increases the reaction efficiency. BSA has also been made one of the necessary components of the PCR kits, which can produce reliable STR profiles (Kreader, 1996; Park, Kim, Yang, & Lee, 2008; Staševskij, Gibas, Gordevičius, Kriukienė, & Klimašauskas, 2017). One of the most important components in the PCR reaction is polymerase enzyme, and by using high efficiency polymerase such as Picomaxx High Fidelity, Bio-Ex-act Short and Ex-Taq-Hot Start polymerase, a reliable amplification reaction and a complete DNA profile can be created from low template of starting DNA material (Hedman, Nordgaard, Rasmusson, Ansell, & Rådström, 2009)

Electrokinetic and fluorescent dye methods for improved LCN technique

The quantity of the product that will be injected into the capillary tubes can be increased by increasing the voltage, or by increasing the time of the electrokinetic injection in the capillary. It has been observed that this strategy can give fluorescence which is equal to more than nine copies of the alleles, hence producing increased peak heights and improved STR profile. By increasing injection time for thirty seconds, and by increasing voltage up to four kilo volts, it has been determined that relatively low stutter ratio is produced leading to an interpretable DNA profile. Different laboratories use different injection times and different voltages for electrokinetic injection depending upon the internal validation of the laboratories (Prinz et al., 2006). It has been observed that along with time and voltage, type of electrophoresis machine and STR kits also play a crucial role in determining the outcome of the DNA profile (Caragine et al., 2009). These various processes not only increase the sensitivity of reaction but also create interpretable DNA profile from low copy number DNA.

Fluorescent dyes play an important role in detection of peaks during capillary electrophoresis. In the past, several dyes have been used for detection of amplified DNA. Fluorescence can be produced on the exposure of UV light. For increasing fluorescence signals, multi-color dye, that is, monomeric and dimeric dye, have been used in the past (McCord, McClure, & Jung, 1993). A fluorescent intercalating dye is used for detection because they bind and intercalate at different sites in DNA to produce fluorescence after absorbing different wavelength of light. Thiazole orange is a good example as it binds to DNA molecule with every 2 bp of DNA. Another approach for increasing sensitivity is to use laser-based fluorescent for the detection of DNA (Andréasson, Gyllensten, & Allen, 2002; Zhu et al., 1994).

Recent advancement in forensic DNA analysis has enabled the use of fluorescence dye-labeled primers in STR profiling. These dyes often give reliable peak-height ratios when optimal amount of template DNA is used. However, when amount of template DNA is very low, the use of fluorescent energy transfer dye-labeled system (ET-16) has been proved helpful. It has been observed that due to high fluorescence-giving property of this system, it can give accurate and reliable peak height ratios of the STR alleles even when template DNA quantity is 62.5 pg. This system proposed that by increasing the quality of the fluorescence labeled primer, reliable STR profiling results can be obtained even from low amount of starting template DNA (Yeung et al., 2008).

Fluorescence signals can also be enhanced by increasing the sensitivity of the instruments. Modern genotyping instruments, specially dedicated for forensic DNA STR purposes, have proved to be more sensitive instruments for fluorescence production as compared to the old ones, when the same amount of template DNA is used. It creates detectable and reliable peak heights even with less amount of starting template DNA (Ensenberger et al., 2010).

Potential risks of LCN typing and challenges

Low copy number (LCN) DNA evidences can arise from variety of sources. A number of handled objects, such as touched surface of weapon associated with a crime, wearing clothes and many other such evidences represent sufficient skin contact to transfer minute quantities of DNA on the contact surface (Wickenheiser, 2002). The increase in the number of cycles in PCR reaction for typing low copy DNA can cause several unwanted artifacts. potentially causing stochastic fluctuation in the final PCR product. When the template DNA is too low, it is possible that primers do not bind properly to their specific locus resulting in deletion of alleles causing allele ‘drop-out’ or amplification of extra or non-specific alleles in the final PCR product causing allele ‘drop-in’. This stochastic effect also leads to imbalance in the peak-height ratios at a given locus which causes immense difficulties in determining genotype at that particular locus (Clayton, Guest,

Urquhart, & Gill, 2004; Walsh, Erlich, & Higuchi, 1992). By increasing injection time and voltage of the electrokinetic injection, the amount of machine artifacts increases. Increase in voltage may also produce sharp peaks in the electrophergram or raised baseline or both which leads to false STR allele calling and wrong genotyping at that locus (Butler, 2011a; Gill et al., 2000; Lucy, Curran, Pirie, & Gill, 2007).

The modified PCR primers which produce high fluorescence signals have been associated with high risks of the detachment of dyes which produces dye blobs. This is inherent artifact of some of the fluorescence based primers, which increases when high fluorescence emitting primers are used (Butler, 2011b). These dye blobs are however easily identifiable by observing their peak pattern as these create more wider peaks appearing in the start of each dye-panel. Another issue concerning with high fluorescence labeled primer is the chances of pull-ups which are created on the same data-point in the DNA profile but in different dye-panel, leading to high percentage of stutters and wrong calling of alleles. In rare cases, use of high fluorescence dye labeled primers or usage of high power of laser may produce peaks appearing in all the panels, a process known as bleed through, causing difficulties in accurate calling of alleles and determining correct genotype profiling (Butler, Buel, Crivellente, & McCord, 2004).

Use of low template staring DNA quantity creates more chances of contaminations. These contaminations can arise from any stage: from collection of evidence to its analysis. Contaminations can also arise from the personnel handling the sample during various stages of the generation of DNA profile, which is the reason for strict requirement of staff elimination database in forensic DNA laboratories that can be used for elimination purposes if required (Sullivan, Johnson, Rowlands, & Allen, 2004). It is true that generating DNA profile from various low template containing DNA evidences helps in solving many forensic cases but It can be difficult to determine accurate DNA profile when working with low copy number template DNA. It has been observed that profile obtained from low quantity of template DNA contains allele drop-in or allele drop-out making it difficult to ascertain results and apply statistical calculations (Curran & Buckleton, 2010; Hansson & Gill, 2017). There is no consistent widespread methodology for allele designations in low template DNA-based profile, however, it has been suggested that repetitive methodologies can help increasing confidence level in DNA profile. In some cases, four repetition of a low template DNA profile is considered reliable and allele appearing twice in these four repetitions can be assigned an allele call. Statistical applications on this profile are also difficult. It has been suggested that use of Random Man Not Excluded (RMNE) or Probability of Exclusion (PE) should not be applied on this profile as there are more chances of allele drop-out and accounts for various artifacts often observed in this DNA profile which can go against the suspect (Bieber, Buckleton, Budowle, Butler, & Coble, 2016; Buckleton & Curran, 2008).

The use of Likelihood Ratio (LR) has been found more satisfying in giving statistical results as encountering artifacts are also calculated when LR is used in calculating statistical calculations. Besides statistical calculations, determining the detection threshold and interpretation threshold is also a problem (Buckleton & Triggs, 2006). It has been suggested that use of detection threshold less than 50 relative fluorescence unit (RFU) is more useful when eliminating the background noise, while for determining the interpretation threshold, 200 RFU is required that will ensure that a heterozygous locus is not mistakenly designated as homozygous. However, exclusive in-house validations are required in forensic laboratories to determine the limit for detection and stochastic threshold at each dye panel, and for each machine they use for various starting DNA template quantities (Gill, Solis, & Curran, 2009).

Mixture interpretation of low template DNA profiles is also problematic. Determination of major contributor and correct designation of homozygosity or heterzygosity at each locus is cumbersome due to allele drop-in, allele drop-out, high percentage of stutter and contaminations. Besides, it is also difficult to determine the number of individuals present in a given DNA sample due to above mentioned inherent problems of amplifying low template DNA sample (Bleka, Storvik, & Gill, 2016; Budowle et al., 2009; Gill et al., 2000).

Conclusion

Forensic DNA evidence has been heavily relied upon and widely accepted in courts for its high statistical weight. Moreover, STR based DNA profile obtained by using these strategies can be difficult to interpret and requires expertise to correctly label STR alleles. Regardless of its potential risks, creating STR profile from low template DNA evidence can provide important information in solving several forensic cases.

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