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Review

The value of Non-CODIS miniSTR genotyping systems in forensic casework in South Africa

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Genetic identity testing is achieved by examining polymorphic regions of DNA. Currently, STR markers are the most commonly used loci for human identification from which several commercial genotyping systems have been developed based on the 13 core CODIS loci. However, identification of degraded or compromised DNA proved to be difficult. Recently, research into genetic typing systems has focused on making use of multiplex polymerase chain reaction (PCR) systems that have a reduced size of the PCR products, to improve the molecular analysis of degraded DNA samples in particular. Non-CODIS (NC) loci were therefore developed to aid the profiling of degraded or compromised DNA samples. This paper reviewed the latest developments and improvements on the non-commercial NC miniSTR genotyping systems. The development and validation of these systems are of great interest to South Africa, to reduce the cost of forensic casework.

Key words: Forensic genetics, MiniSTR, Non-CODIS, allele frequency, population genetics, South Africa.

INTRODUCTION

Despite with a slight decrease, the latest statistics released by the South African Police Service showed that more than 2 million serious crimes were reported in South Africa, during the 2010/2011 financial year (http://www.saps.gov.za/statistics/reports/crimestats/2011/crime_situation_sa.pdf). These serious crimes included murder, attempted murder, assault with the intent to do grievous bodily harm, common assault, sexual offences, aggravated robbery and common robbery. The high crime rate in the South Africa is due to a number of complex factors, including the turbulent history during the Apartheid era, where the policy of racial segregation and social injustice has brought high levels of crime and violence. A number of measures are being implemented to address this challenge that is facing the South African society. In most instances, measures that could address this problem are beyond the scope of solutions that can be offered by biotechnology. Despite this, a contribution can be made in at least one area, which is forensic genetics (Leat et al., 2004). By developing robust DNA-based identity testing systems, biotechnology can assist in the identification of perpetrators of violent crimes (Leat et al., 2004).

The South African Police has been using DNA-based identity testing systems to assist in forensic casework in the country. This includes mainly the use of commercial systems for the analysis of autosomal genetic markers (Leat et al., 2004). Even though autosomal genetic marker systems are well established, difficulty is still faced when working with degraded or compromised DNA samples. Sometimes, the postmortem identification cases involve advanced states of decomposition. This was generally the case when trying to identify individuals of crimes from the apartheid era (Davison et al., 2008). Recently, research into genetic typing systems has focused on making use of multiplex polymerase chain reaction (PCR) systems that have a reduced size of the PCR product, to improve the molecular analysis of degraded DNA samples in particular. A great interest was also shown in the development and validation of non-commercial genotyping

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systems, to reduce the cost of forensic casework.

GENETIC IDENTITY TESTING

Genetic identity testing involves identifying patterns of genetic material that are unique to almost every individual (Saad, 2005). It takes advantage of the 0.3% DNA sequence variations in the human genome to distinguish between individuals (Butler, 2005). Most human identity testing is performed using DNA markers on the autosomal chromosomes, and gender determination is done with markers on the sex chromosomes (Butler, 2005). A number of polymorphic genetic elements could be used in human identity testing. These include single nucleotide polymorphisms (SNPs) and micro-satellites (Leat et al., 2004). SNPs are the most abundant form of genetic variation in the human genome and occur as a result of a single nucleotide substitution (Sobrino et al., 2005). SNPs mutate is relatively slow in comparison with its micro-satellite counterparts and therefore require a substantial number of simultaneous analyses in order to discriminate between individuals (Leat et al., 2004). Micro-satellites or short tandem repeats (STRs) occur as a result of repeating DNA sequences which is normally ~2 to 6 bp in length and are normally repeated ~5 to 20 times (Chung et al., 2004; Rudin and Inman, 2002; Schoske et al., 2003). STRs do not only show differences in the length and the number of repeating units, they also differ in their repeat patterns (Butler, 2005). Some STR markers contain simple repeats wherein the repeat units have identical length and sequence, whilst others contain compound repeats where the repeating unit contains two or more adjacent simple repeats. Other STR markers can contain complex repeats where the repeat unit is made up of a number of repeat lengths and may contain variable intervening sequences (Butler, 2005). Furthermore, other alleles may contain incomplete repeat units and are known as microvariants (Butler, 2005). An example of a microvariant is the allele 9.3 at the TH01 locus. It contains nine complete AATG repeat units and one incomplete repeat wherein an adenine is missing (Puers et al., 1993). For STR analysis, primers are designed from sequences known to flank the STR locus. This enables the amplification of alleles whose sizes vary by the number of repeating units they contain (Strachan and Read, 2004).

STR markers and their usage in forensic DNA typing

Forensic samples can be very challenging to work with. Sexual assault cases will most likely contain a mixture of DNA from both the victim and the suspect. Remains may have been exposed to a number of different factors such as fire, water, or environmental factors such as bacterial and fungal contamination, insects and growth of microorganisms (Clayton et al., 1995; Budimlija et al., 2003; Lessig et al., 2006; Bender, 2004). All these factors play a contributory role in making it difficult to correctly identify victims. STR loci are the most commonly used loci for human identification (Chung et al., 2004; Butler, 2005; Butler et al., 2003). This is because these loci are highly polymorphic, require minimal template DNA to facilitate genotyping and they possess a narrow size range, thereby making it more ease when multiplexing (Hummel et al., 1999; Butler, 2005; Chung et al., 2004; Opel et al., 2006). Dinucleotide, trinucleotide and tetra-nucleotide STR loci have been used as markers in linkage studies and in the building of the human genetic map (Fornage et al., 1992; Edwards et al., 1991). Tetra-nucleotide loci have however been favourably usage as forensic markers since they are highly polymorphic and display heterozygosity values greater than 0.90 (Walsh et al., 1996).

Autosomal CODIS STR loci

The FBI developed a database of STR markers known as the COmbined DNA Index System (CODIS), which comprised of 13 core STR loci. These 13 CODIS loci are: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S359, D18S51 and D21S11 (Butler, 2005). To date, these loci are generally used for identification of humans (Butler, 2006). These 13 loci can be amplified simultaneously and were thus developed into commercial genotyping kits. This permits the construction of DNA databases that can be used and shared worldwide. The commercial genotyping kits have proved to be very successful in certain forensic casework samples, however problems have been reported when these kits were used to analyze degraded DNA samples or DNA samples which had PCR inhibitors present. To improve the performance of the CODIS genotyping systems, the primer binding sites of the CODIS loci was redesigned to produce shorter amplicons. Smaller amplicons were achieved by moving the primers closer to the STR repeat region, thereby generating shorter amplicon fragments (Figure 1). The newly redesigned primers were combined into multiplexes and referred to as miniplexes. Each locus was allocated a single dye colour. Thus, one drawback of these miniplex systems is that unlike the normal STR commercial kits where 13 loci can be simultaneously amplified, four to five amplifications are required to facilitate typing of all the core loci (Butler et al., 2003) and in some instances this proved to be counter productive as was experienced by Parsons et al. (2007). The miniSTR primer sets were able to produce full genetic profiles in the majority of the samples containing degraded DNA, however there were still some instances where only partial profiles could be obtained (Hummel et al., 1999; Butler et al., 2003; Kleyn, 2009; Romano et al., 2006). The underlying reason for this is that not all the

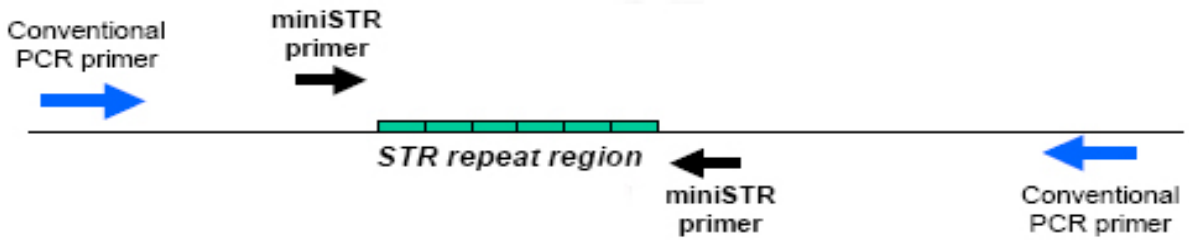

Figure 1. Diagrammatic representation of miniSTR primers. MiniSTR is a reduced sized amplicon of the conventional PCR primers. MiniSTR facilitates high recovery of information from degraded DNA samples (Coble and Butler, 2005).

CODIS loci could be successfully reduced to enable sufficient reduction in product size that would enable it to successfully amplify degraded DNA. For example; the locus FGA contains large allele ranges, D7S820 and D18S51 contains flanking regions, which are not amenable to redesigned primers (Coble and Butler, 2005; Butler et al., 2003). To overcome these limitations, a comprehendsive project was undertaken by Hill and co-workers (2008) to search for new markers that produce shorter amplicons.

Non-CODIS miniSTR LOCI

Hill et al. (2006, 2008) and Coble and Butler (2005) characterized 26 novel miniSTR loci to complement the 13 CODIS loci. The development of these loci facilitated the use of additional chromosomal locations that could be vital in the analysis of degraded DNA samples, as well as missing person's investigations and parentage testing especially in instances where a limited amount of reference samples were available (Hill et al., 2006; 2008; Goodwin et al., 2004; Ricci et al., 2007). The NC loci were preferentially designed on chromosomes that differed from the 13 CODIS loci, but in instances where NC loci and CODIS loci occur on the same chromosome, they were placed approximately 50 Mb apart, thereby eliminating linkage (Hill et al., 2008). Figure 2 illustrates the chromosomal position of NC loci in relation to CODIS loci. All candidate loci primers were designed as close as possible to the repeat regions, in some instances, very close to the repeat, to allow for the smallest possible amplicons. Amplicon sizes ranged between 50 and 150 bp (Hill et al., 2008).

Asamura et al. (2007) developed miniSTR systems which contained NC loci for the analysis of both artificially degraded and degraded forensic casework samples. In both instances, results from miniSTR systems were compared with results obtained from a commercial kit (Asamura et al., 2007). Individually, the miniSTR systems produced more conclusive results in comparison with the commercial kit. However, combining results from both systems (miniSTR system and the commercial kit) proved to be more effective for forensic purposes. Ultimately, this proves that the NC loci can be used to complement or extend any existing genotyping system where conclusive results cannot be obtained when using existing genotyping systems.

DEVELOPMENT AND VALIDATION OF NC-MINISTR GENOTYPING SYSTEMS IN SOUTH AFRICA

A recent study aiming to develop and validate a six Non-CODIS miniSTR genotyping system, and to determine its suitability for forensic casework in South Africa, has recently been completed (Abrahams et al., 2011; Abrahams and Benjeddou, submitted for publication; Abrahams and Benjeddou, in preparation). The work carried out in this study forms part of a more comprehensive project aiming to develop and validate non-commercial miniSTR genotyping systems suitable for forensic casework in South Africa in particular, and in the sub-Saharan Africa region in general. In developing countries such as those in the African continent, the use and development of noncommercial genotyping systems is extremely important to cut the cost of forensic casework. The development of these genotyping systems will help in the forensic casework involving DNA samples and in particular those which are highly degraded. These systems are indeed valuable in a variety of scenarios including complex paternity cases, missing persons work and mass fatality disasters. This includes missing person's work as a result of past political violence during the apartheid era, as well as current domestic violent crime in South Africa.

In the above-mentioned project, and after the successful implementation of the genotyping system in the laboratory, allele size range was determined for each of the loci and allelic ladders were constructed. The entire repeat regions of the six loci under investigation were successfully sequenced. Consequently, allele repeat number, structure and observed size were determined for each locus. An internal validation study of the six Non-CODIS miniSTR genotyping system was conducted following the SWGDAM guidelines (Abrahams and Benjeddou, in preparation). A comprehensive population study, covering five population groups from South Africa was also carried out (Abrahams et al., 2011; Abrahams

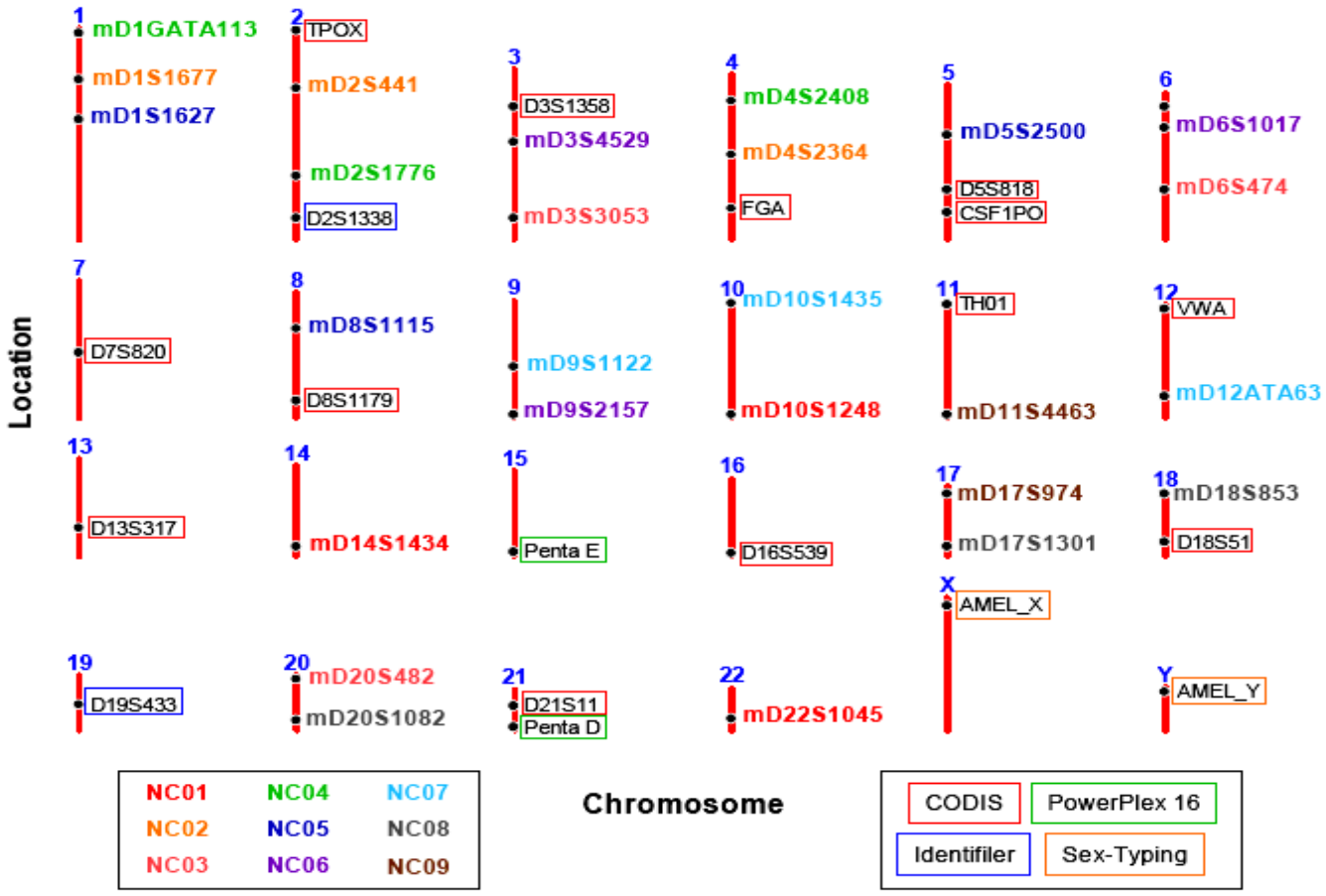

Figure 2. Diagrammatic representation of the Non-CODIS loci. chromosomal position of NC loci in relation to the 13 CODIS loci (Hill et al., 2006).

and Benjeddou, submitted for publication). The genotyping system produced consistent, accurate and precise genetic profiles for low concentrations of template DNA. When analyzing mixed DNA samples, successful differentiation of minor and major DNA components was identifiable. Amplification products were observed in non-human DNA studies but in all instances, complete genotype profiles were not obtained (Abrahams and Benjeddou, in preparation). Allele frequencies and forensic parameters were determined for the system in the five South African population groups (Afrikaner, Asian-Indian, Mixed Ancestry, Xhosa and Cape Muslim) (Abrahams et al., 2011; Abrahams and Benjeddou, submitted for publication). No deviation from Hardy-Weinberg equilibrium was observed in any of the populations, except in the Mixed Ancestry population for the locus D9S2157 (Abrahams et al., 2011; Abrahams and Benjeddou, submitted for publication). Furthermore, all populations displayed a high power of discrimination and a high power of exclusion. The six Non-CODIS miniSTR genotyping system has shown a good potential to aid in the analysis of degraded DNA samples. This system can be further improved by including additional loci. Even in its current form, it can certainly provide additional discrimination in complex paternity and/or missing person cases.

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