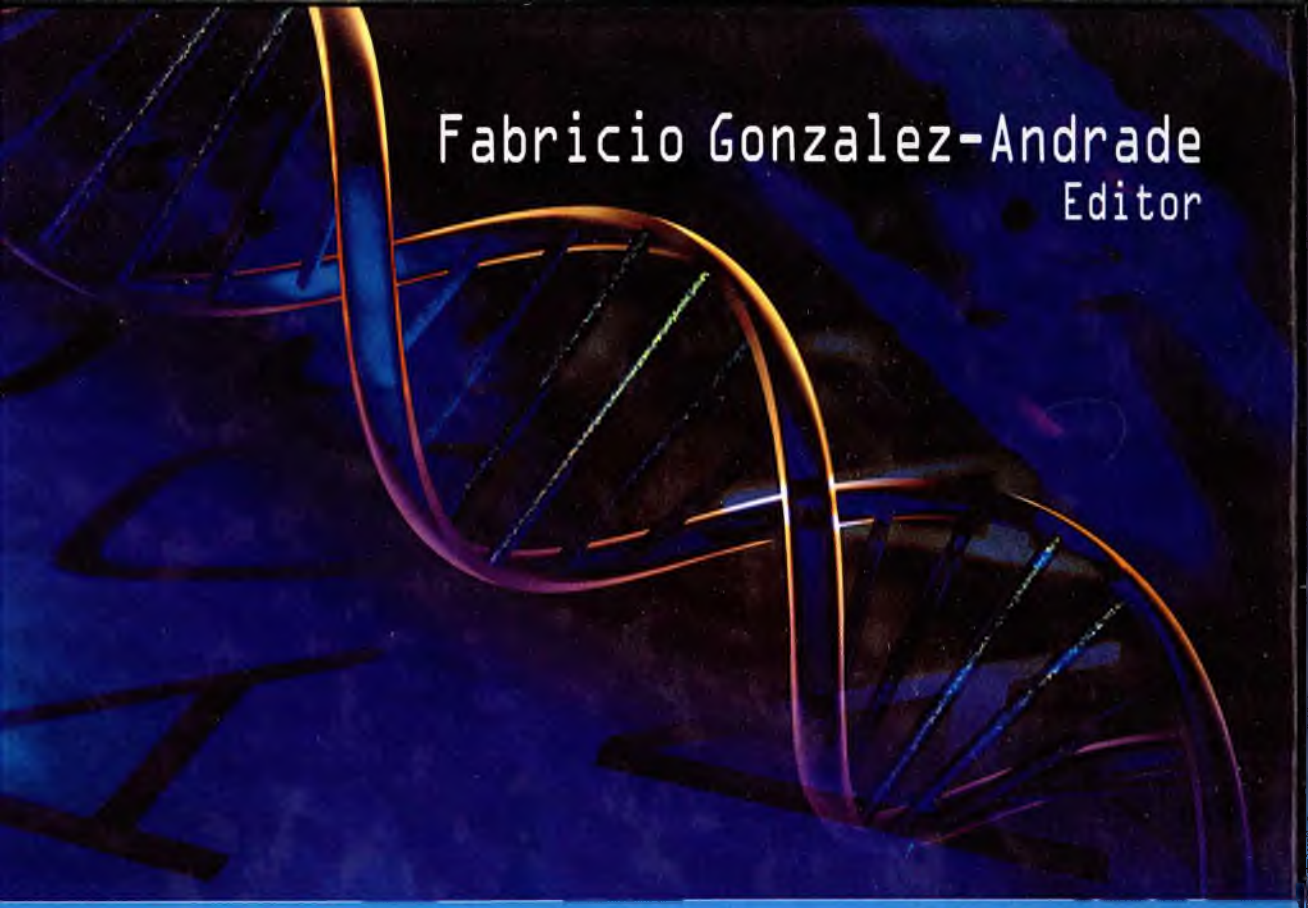

Fabricio Gonzalez-Andrade
Editor

Forensic Genetics Research Progress

NOVA

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In: Forensic Genetics Research Progress
Editor: F. Gonzalez-Andrade

ISBN: 978-1-60876-198-2

Chapter 7

ANALYSIS OF REDUCED SIZE STR AMPLICONS AS TOOLS FOR THE STUDY OF DEGRADED DNA

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ABSTRACT

Polymorphic Short Tandem Repeats (STRs) markers have become a very useful tool in forensic analysis of human DNA. Multiplex PCR amplification of STR loci enables to obtain genetic information from almost any source of biological material. However, some forensic evidences, can be so extremely degraded (e.g. certain mass disasters, old and bad preserved remains, etc) that STR typing is unsuccessful. In these situations DNA typing can be challenging and it could be difficult to obtain quality profiles (ej: loss of signal of larger sized loci is frequently observed as a result of DNA fragmentation, etc.). Recent efforts have focused on the use of nuclear Single Nucleotide Polymorphism (SNP) markers as an alternative and more recently, on reducing the size of STR markers (miniSTRs). The development of miniSTRs is accomplished by simply moving the primer binding sites closer to the STR repeat region, and creating DNA fragments that are shorter than traditional STR markers. MiniSTRs markers have shown to be very successful at recovering DNA profiles from highly degraded samples The possibility of obtaining nuclear MiniSTRs profiles corresponding to conventional STR markers is also considered a great advantage as most of the National intelligence DNA banks are based on them. In this chapter we will review the development and use of miniSTRs loci in forensic genetics from its first application in the analysis of Branch Davidian fire in Waco to its incorporation in commercial kits. We will not only focus on autosomal

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miniSTRs but also on the recent development of miniY-STRs and miniX-STRs. Advantages and disadvantages of these markers in Forensics will be considered and compared to those of traditional STR markers. Finally, we will review potential applications and future perspectives of their use in Forensic Genetics.

INTRODUCTION

Polymorphic Short Tandem Repeats (STRs) markers have become a very useful tool in forensic analysis of human DNA. Multiplex PCR amplification of STR loci enables to obtain genetic information from almost any source of biological material. However, it is common in criminalistics to find forensic evidences that are so extremely degraded (e.g. certain mass disasters, old and bad preserved remains) that STR typing is unsuccessful. During the decomposition of a sample, DNA molecules are gradually degraded in small fragments by the action of endogenous nucleases. The degradation process depends on environmental conditions, such as temperature, humidity, pH, soil chemistry or UV radiation, which can cause molecular damage on the DNA over time, through hydrolysis and oxidation processes. Degradation will limit the amount of amplifiable DNA available, as DNA will be fragmented in small size fragments, and just few of them will have the complete target sequence to be typed. In addition, there can be PCR inhibitors such as humic acid or derived degradation products, coextracted with the DNA, which can impede the PCR reaction (Fondevila et al. 2008).

In these situations it is frequent to observe a loss of signal of larger sized loci, due to DNA fragmentation or, in other cases, to the presence of inhibitors (see Figure 1A). The progressive loss of signal, as size locus increases, can be seen in the electropherogram as a decay curve. The peak height is inversely proportional to the amplicon length, so larger loci will have lower signal, even falling below the detection threshold (see Figure 1B). As a result, degraded samples often result in partial genetic profiles with allele and/or complete locus dropout (Cotton et al. 2000, Wallin et al. 1998).

DEGRADED DNA TYPING

Forensic laboratories have faced the problem of typing degraded DNA samples in different ways. When samples are so degraded that STR typing methods are ineffectual, one possible approach to obtain results is to analyze the mitochondrial DNA (mtDNA) hypervariable regions (Stone et al. 2001, Bender et al. 2000). As the mitochondrial DNA is present in a high number within cells, with hundreds or thousands of copies per cell, there is a high likelihood of success in typing degraded samples. Furthermore, the haploid and maternal transmission between generations facilitates the comparison among maternal relatives, since they should have an identical mtDNA. However, mtDNA analysis has the disadvantage of being a time-consuming and labor-intensive process. But the principal limitation in forensic mtDNA testing is the difficulty to differenciate among individuals who share common HVI/HVII haplotypes, resulting in a low power of discrimination (Just et al. 2004, Parsons et al. 2001).

Recent efforts have focused on studying shorter regions of DNA, such as Single Nucleotide Polymorphism (SNP) or smaller STR markers (miniSTRs), as alternative approaches to analyse degraded DNA (Dixon et al. 2006). The utility of autosomal (Whitaker et al. 1995), mitochondrial (Quintans et al. 2004) and Y chromosomal SNPs (Bouakaze et al. 2007) have been proved in challenging samples typing.

SNPs markers offer a good alternative to STR analysis because of their small size (<150bp), abundance and possibility to automation (Odriozola et al, 2009). But unfortunately, SNP analysis has the setback of requiring the study of a high number of loci (45-50) to reach match probabilities comparable with STR multiplexes (Butler et al 2007).

Reducing the size of STR (miniSTR) to amplicon sizes less than 200 bp has become a successful method of typing small fragments of DNA. It is especially appropriate for degraded DNA evidence, for example from mass disasters or anthropological remains.

The development of miniSTRs is accomplished by simply moving the primer binding sites closer to the STR repeat region, and creating DNA fragments that are shorter than traditional STR markers (Figure2). It is important to have in mind that repeat information is independent of amplicon size, which will be defined by primers position.

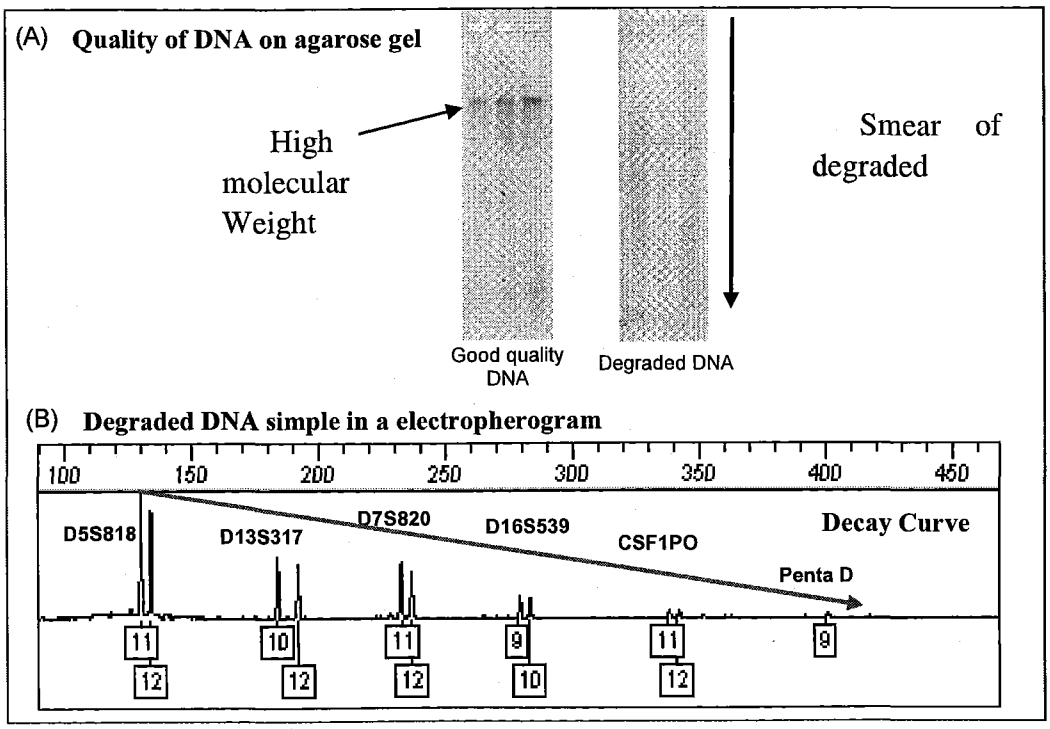

Figure 1. (A) Quality of DNA sample on agarose gel. Degraded DNA is fragmented into small pieces that will be seen on the gel as a smear. (B) Decay curve: progressive loss of signal as size locus increase, the larger loci often fall below the detection threshold. Modified from Butler J, 2005.

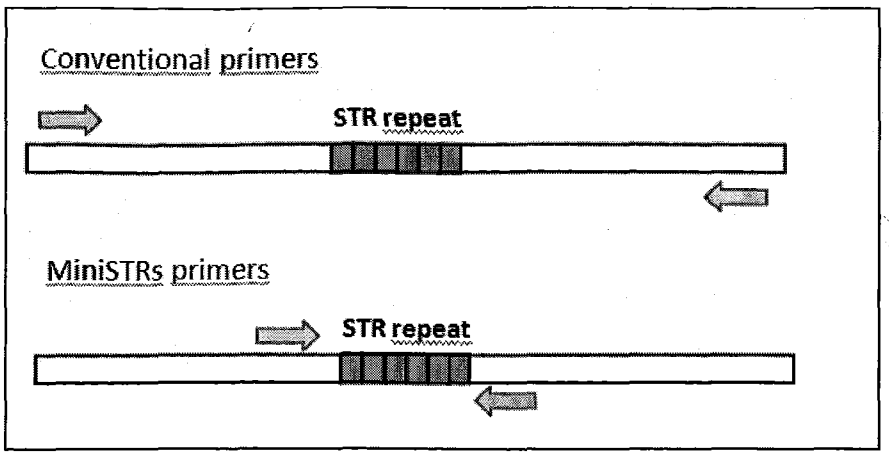

Figure 2. Methodology used to reduce amplicons.

Recently there has been some debate about which new markers should be implemented to increase the success of typing degraded DNA: conventional STRs, miniSTRs, or SNPs. The EDNAP and ENFSI groups concluded that miniSTR analysis, in general, is the most effective approach for degraded DNA (Dixon et al. 2006).

MINISTRS TIMELINE

The first evidence that smaller STRs worked better than larger loci was the successful analysis of severely degraded samples taken from the scene of the Branch Davidian fire in Waco (Texas) (Whitaker et al. 1995).

Afterwards some authors started to generate new primer pairs of STRs in monoplex reactions which showed an improvement in the efficiency of amplification, especially in degraded samples (Yoshida et al. 1997; Ricci et al. 1999).

Wiegand and Kleiber (2001) demonstrated that highly degraded DNA, as well as very low amounts of DNA, could be more successfully typed using some new redesigned PCR primers. These new primers were closer to the STR repeat compared to the established sequences that generated longer amplicons for the same loci. Later, these results were confirmed by a study where a multiplex reaction for the TH01, TPOX, CFS1PO, and vWA loci was performed with newly designed pair of primers (Tsukada et al. 2002).

Butler et al. (1998) started to work in mass spectrometry typing and multiplexing using small STR amplicons. New primer sets for Y chromosome and CODIS STR loci were designed and tested using conventional capillary electrophoresis instrumentation, showing reliable STR typing (Ruitberg et al. 2000).

Following the World Trade Center attacks, it quickly became evident that traditional methods for DNA typing were not likely to be fully successful in identifying all of the recovered remains. Thus, a big effort was made to improve and develop new technologies of DNA analysis to identify the huge number of victims (Holland et al. 2003). Indeed, the short STRs methodology was accelerated to be used in aid of victim's identifications.

Table 1. Miniplex systems described.

Author/s	Name	Included markers
Schumm et al. 2004 Bode Plexes were used in the WTC victim's analysis (2002) by the Forensic Institute of NYC.	BodePlex 1:	D13S317, D21S11, D7S820, D16S539, CSF1PO
	BodePlex 2:	TPOX, FGA, D7S820, D18S51
Butler et al. 2003 In D3S317, D5S818 y VWA there is no concordance with commercial systems due to the presence of delections and dimers formation (Drabek et al. 2004)	Miniplex 1	CSF1PO, TH01, TPOX
	Miniplex 2	D5S818, D8S1179, D16S539
	Miniplex 3	FGA, D7S820, D21S11
	Miniplex 4	VWA, D13S317, D18
	Miniplex 5	Penta D, Penta E, D2S1338
	Big Mini	CSF1PO, FGA, TH01, TPOX, D7S820, D21S11
Chung et al. 2004	Miniplex 2	D5S818, D8S1179, D16S539
	Miniplex 4	VWA, D18S51, D13S317
	Miniplex 5	Penta D, Penta E, D2S1338
	Big Mini	Multiplex 1: TH01, TPOX, CSF1PO Multiplex 2: FGA, D21S11, D7S820
Coble et al. 2005 Development of potential miniSTRs, candidates with more heterozigosity which produced < 125 bp amplicons.	Miniplex 1	D10S1248, D14S1434, D22S1045
	Miniplex 2	D1S1677, D2S441, D4S2364
Gill P et al. 2006 EDNAP/ENFSI recommendations for the development of new miniSTRs.	Miniplex 1	D10S1248, D14S1434, D22S1045
	Miniplex 2	D12S391, D1S1656, TPOX
Wiegand et al. 2005	Pentaplex	Amelogenin, TH01, D3S1358, VWA, FGA
	Multiplex 2	TH01, D3S1358, VWA, FGA
	Blue kit	D3S1358, VWA, FGA
Butler et al. 2005a	Mini SGM	TH01, Amelogenin, FGA, D18S51, D16S539, D21S1138
	Mini NC01	D10S1248, D14S1434, D22S1045
Hill et al. 2006. Development of 27 new MiniSTR loci for improved analysis of degraded DNA samples. Poster B105 at AAFS Congress, Seattle, 2006.	NC01	D10S1248, D14S1434, D22S1045
	NC02	D4S2364, D2S441, D1S1677
	NC03	D3S3053, D6S474, D20S482
	NC04	D1GATA113, D2S1776, D4S2408
	NC05	D1S1627, D5S2500, D8S1115,
	NC06	D3S4529, D6S1017, D9S2157
	NC07	D9S1122, D10S1435, D12ATA63
	NC08	D17S1301, D18S853, D20S1082
	NC09	D6S1027, D17S974, D11S4463
Hill C et al. 2006 Loci rejected and reasons for not considering them.	D9S324: allelic displacement, presence of complex repetitions. D10S1430: presence of complex repetitions. D10S2327: profiles with three and four alleles. D14S297: poor heterozigosity. D15S817: profiles with three and four alleles.	

Butler and McCord developed five multiplex assays that covered all of the CODIS STR loci as well as D2S1338, Penta D, and Penta E, and they termed such systems as "miniSTRs" or "miniplexes" (Butler et al. 2003) (Table 1). They combined two sets of primers to develop the "Big Mini", which was used by the Office of Chief Medical Examiner of New York (NY OCME). However, the miniSTR concept still needed to be further studied to increase the sensitivity and be capable of working robustly on an industrial scale in order to process thousands of samples from the WTC site. Based on this strategy two "BodePlexes" were developed and used on samples from the WTC investigation (Schumm et al. 2004).

Since then, several miniplexes have been developed and evaluated to improve the strategy (Chung et al. 2004; Drabek et al. 2004; Coble et al. 2005; Opel et al. 2006; Hill et al. 2008).

Recently, the EDNAP and ENFSI groups recommended the implementation of miniSTRs as the way forward to increase both the robustness and sensitivity of forensic DNA analysis (Gill et al. 2006b).

An important progress for miniSTR technology was the development of the "MiniFiler" kit by Applied Biosystems, a 9-plex kit that proved its concordance with conventional STR typing kits (Hill et al. 2007).

Pros and Cons of MiniSTRs

As it was previously commented, miniSTRs markers provide a very useful tool for analysing degraded DNA samples. Their small size increases success rate of degraded DNA analysis, allowing the recovery of valuable information from casework samples, which would gave negative or very partial results if analysed with STR markers (Wiegand et al. 2001, Tsukada et al. 2002, Coble et al. 2005, Grubwieser et al. 2006)

The usefulness of miniSTR assays has been confirmed in intra- and inter-laboratory studies involving degraded bones and aged blood and saliva stains (Opel et al. 2006, Butler et al. 2003). In all cases, miniSTR markers gave higher success rates in recovering information than conventional STR kits. Their efficacy has also been validated in telogen hairs, samples frequently found in crime scenes (Hellmann et al. 2001). Until the development of miniSTRs markers, it was complicated to obtain DNA profiles from these samples, because nuclear DNA from keratinized cells was highly degraded (about 100 bp in size), and as result, the use of conventional STRs would show a low success rate or even fail.

MiniSTRs loci also provide a very sensible approach that permits to obtain genetic profiles from low amounts of DNA. Whereas traditional STR testing works best with about 1-2 nanograms of DNA, miniplexes can get valuable results from 0.25-0.5 nanograms of DNA, or even from less than this quantity of DNA. Furthermore, the new miniSTR systems will also significantly increase the success of amplification of samples containing PCR inhibitors, such as humic acids or heme groups, which adversely affect the STR multiplex systems (Eisenberg et al. 2007).

The compatibility of several miniplexes with commercial STR kits has been tested to check possible discrepancies due to allele dropouts. A high concordance of results between the two methods was established (Drabek et al. 2004, Hill et al. 2007). This compatibility with STRs will allow the comparison between miniSTRs, and CODIS and international databasing standards, reducing the possibility of discrepancies.

Actually these new markers are not meant to replace the current CODIS loci, but complement them, especially in cases where additional genetic information is needed. For example, in situations where there are closely related individuals involved, such as complex paternity cases (e.g., incest) or mass disasters, the use of miniSTRs will increase the discriminating power of the multiplexes. In fact, the implementation of miniSTRs will augment both the robustness and sensitivity of analysis, providing additional statistical support for an association. Indeed, the kit Minifiler (Applied Biosystems) offers highly discrimination results with probability of Identity values of less than 1×10^{-10} for US populations.

Moreover, miniSTRs constitutes a good alternative to mtDNA typing in degraded samples, as the results obtained by mtDNA analysis cannot always be compared with DNA profiles from routine casework or from databases.

Furthermore, new laboratory equipment or additional staff training is not necessary to work with miniSTRs, because the same methodology as STRs is used. Consequently, the implementation of these markers in laboratories will not cause further technical or economical difficulties. Besides, alternative technologies such as high-speed microfluidic and time-of-flight mass spectrometric can be applied to these miniplexes (Butler et al. 1998, 2001, Schmalzing et al. 1997).

Unfortunately, the miniSTRs have some disadvantages. A major inconvenient is the small number of miniSTRs that can be coamplified in the same multiplex reaction. As a result of the size reduction, the different miniSTR loci have a similar size range (~100bp), causing their overlapping within the same fluorescent dye lane. For this reason, there will be typically one miniSTR locus per dye color (instead of the 4 or more loci included in STRs multiplex). This will decrease the power of discrimination of multiplexes; for example, a 6-miniplex with 100% amplification success will give less genetic information than a 16-plex where the six largest amplicons loci have dropped out (Parsons et al. 2007). One way to overcome this limitation is to combine the typical 5-dye fluorescent system with the addition of non-nucleotide linkers. This will allow the simultaneous amplification and efficient separation of the different miniSTR loci, without the risk of overlapping.

Another important limitation is that not all STRs can be reduced in size. For example, some of the CODIS core loci have large allele ranges that make not viable the design of smaller amplicons (e.g. FGA, with a range size of 156 pb). In other cases, it is not possible to bring primers as close as possible to the STR repeat region, because the flanking sequences contain polymorphic nucleotides, partial repeats, mononucleotide repeat stretches, or insertions/deletions that could prevent stable primer annealing. This is the case of D7S820 locus that contains a poly-T stretch, located 13 nucleotides downstream of the core repeat, and that can have eight, nine, or ten T's. Consequently, the reverse primer for this marker has to be outside the poly-T stretch to retain this variation and permit full concordance with commercial STR kit results. The design of primers for other loci also shows similar problems. FGA has a partial repeat and the mononucleotide repeat stretch "TTTC TTCC TTTC TTTTTT" downstream of the core repeat. In order to avoid this problematic region, the reverse primer for FGA binds 23 nucleotides away from the end of the repeat, limiting the size reduction (Butler et al. 2003).

Another point to be considered is the fact that different primers are used in STR and miniSTR typing, so possible genotyping inconsistencies can be observed due to allele dropout. Indeed if some point mutations, insertions or deletions happened in the STR flanking

regions, but outside of the miniSTR primer binding sites, STR primer annealing could be prevented. Consequently an allelic dropout could appear, causing potential discrepancies with miniSTR results, since the individual would be erroneously considered homozygous for this locus. For example, loci D8S1179 and vWA have some polymorphic nucleotides in the flanking region that will affect STR amplification causing allele dropout, but it will not have effect on miniSTR typing (Alves et al. 2001, Han et al. 2001, Budowle et al. 2001).

Another problem of concordance can be seen in D13S317 locus typing. This locus has a four-base deletion of TGTC that is 24 bases downstream from the core. The miniSTR reverse primer is located between the repeat region and the potential deletion sequence, thus its amplification will not be affected by the mutation. On the contrary, STR primers are located outside of the deletion region, so in presence of the deletion, the amplification product will be 4bp shorter than in miniSTR typing (Boutrand et al. 2001).

A different problem to solve is the potential contamination derived from the high number of cycles used in the amplification (28-35 cycles). Since such a small sample is being amplified, this can be easily contaminated by exogenous DNA. Moreover, it is important to consider that despite the high sensitivity of the method, PCR inhibition problems can occur. Although shorter fragments are supposed to amplify more efficiently and be more resistant to Taq polymerase inhibition, the presence of inhibitors can also affect the PCR (Parsons et al. 2007).

NULL ALLELES IN MINISTRS

As we mentioned above, occasionally, sequence variation (point mutations or indels) could occur in the flanking region of commonly used STR loci. As result, the primer annealing to the STR locus during amplification can be prevented, resulting in a null allele. This phenomenon can result highly problematic in the interpretation of results, since a heterozygote for a detected allele and a null allele can be erroneously considered as homozygote for the detected allele. Other possible causes of microsatellite null alleles are the preferential amplification of short alleles (large allele dropout) or PCR failure due to low quality or quantity of DNA (Wattier et al. 1998, Wanderler et al. 2003).

There are various approaches to estimate the frequencies of null alleles. Chakraborty (1992) and Brookfield (1996) estimated null allele frequencies through statistical analysis of STR typing data, assuming that the heterozygote deficiency is caused by null alleles and not by other genotyping errors. They predicted potential null alleles comparing the observed and expected heterozygosities based on Hardy–Weinberg (HW) equilibrium. The statistical methods are indirect approaches to the detection of microsatellite null alleles. In fact, the best approach is a molecular study by directly sequencing the null allele.

Understandably the presence of null alleles will have a strong impact in population genetic databases, as they can bias allele frequencies and produce an apparent deficiency of heterozygosity (or excess of homozygosity), compared with Hardy-Weinberg expectations. This can be solved by doing less rigorous searches in databases.

On the other hand, null alleles will also create difficulties in forensic studies since they can lead to incorrect exclusions of samples that are from the same source but appeared to be

different. Indeed, they can cause false parentage exclusion if the parent and child in question show an apparent homozygosis for different alleles (Dakin et al. 2004).

After detecting a null allele in a STR locus, there are several possible solutions to correct it, such as discarding the problematic locus, redesigning the primers, using degenerate primers (that include the problematic primer binding site variant), or adjusting allele and genotype frequencies based on the estimated null allele frequency (Butler 2005b).

It is important to consider all known information about flanking sequence variation in the design of primers to avoid possible null alleles. Furthermore, primer concordance studies are indispensable to look for possible discrepancies between commercial kits and miniplexes.

Standardization of STR Loci

EDNAP and ENFSI have collaborated to standardize DNA profiles in Europe, so comparisons between laboratories become straightforward (Gill et al. 2006a). Most of this standarization effort has focused on STR loci (Chakraborty et al, 1999; Gill, 2002; Butler, 2006) and as a result they have become nearly the gold standard in Forensic Genetics. Intelligent banks for Criminal purposes have been built on a consensus set of 13 core STR markers (CODIS system) established by the FBI Laboratory (Budowle et al, 1998). This core includes the 13 STR loci, CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11.

Nowadays a number of different STR multiplexes are in use for Forensic casework. In fact, even though there are seven Interpol STR loci established (Schneider et al. 2001; Martin et al. 2001), for instance, most European laboratories show a much higher degree of compatibility because they use the same multiplex systems as AmpFlSTR® SGM Plus, AmpFlSTR® Identifiler, or PowerPlex 16® System. However, it is still necessary to modify existing multiplexes to increase success rates in degraded DNA typing (Schneider et al 2004).

The rapid implementation of new loci in National databases would be possible as a result of the high level of scientific and technical standardisation reached in forensic laboratories. Nevertheless, due to the differences between countries in the choice of loci included in National DNA databases it is important to adapt new multiplexes to national requirements. The most practical strategy would be to design multiplexes that incorporate new loci, in addition to those currently used, rather than discarding the existing loci. Furthermore, if miniSTRs were included in the multiplexes already established higher success rates could be achieved with degraded samples.

Other issues to solve are legislative and political considerations. In fact, in some countries this would mean to alter the existing legislation to be able to include new markers.

Recently, it has been proposed to connect database laboratories using virtual networks, instead of using a centralised database. With this system, each country would maintain control over their databases and access to other databases information according to local legislative requirements. Additionally, searches would not be limited to the Interpol core loci.

Databases should be dynamic and keep up with the scientific development of Forensic Genetics. Thus, new genetic markers could be changed or included if one of these reasons exists:

1. To improve the system's discrimination power.

2. To improve the analytical sensitivity of low copy number.
3. To improve the robustness or quality of the results.

Since national DNA databases have been built using different multiplexes, it is not realistic to suggest the retirement of existent loci in favour of new ones. For this reason, the investigation of new core loci has been proposed, making possible to laboratories to expand their multiplexes while maintaining their existing set of STRs. These new core loci will be decided by the EDNAP group in collaboration with the ENFSI group. Afterwards some less value loci could be retired, even though the information after their analysis stayed as a remnant in databases.

ENFSI/EDNAP Recommendations about the Incorporation of New MiniSTRs

1. MiniSTRs will be adopted as the approach to increase the robustness and sensitivity of DNA analysis.
2. Core loci in national databases will be retained and transformed into miniSTRs by re-engineering, moving the primers closer to the repetitive region (Drabek et al. 2004; Hellman et al. 2001).
3. It is recommended the adoption of a new multiplex called Miniplex 1 (Table 2), with D12S1248, D22S1045, D2S441 markers between 70 and 125 bp. Also, DS441 instead of D14S1434, because the second one has a low discrimination power in comparison with the other loci (D2S441, PD = 1/2500; D14S1434, PD = 1/7).
4. A secondary loci list which could be converted into miniSTRs has been compiled. This multiplex 2 includes D12S391, D1S1656, and TPOX markers. The first ones are miniSTRs between 120 and 180 bp. These loci are not in consensus, but they are recommended as additional loci to increase the multiplex size (Hellman et al. 2001; Gill et al. 1998; Shigeta et al. 2002).
5. The new multiplexes should be similar in sensitivity to that already existent, which means they should be able to detect DNA in quantities less than 250 pg, using standard PCR amplification methods. The main purpose is to facilitate partially degraded DNA detection, taking into account the correct balance of multiplex size versus its efficiency.
6. It is suggested that ENFSI/EDNAP evaluate new multiplexes following the proposed recommendations.

Table 2. Miniplex I (recommended by ENFSI/EDNAP) is derived from miniplex NC01 developed by NIST (Coble et al. 2005; Butler et al. 2003).

STR	**Repeti-tion**	**Motif**	**Chromosomic position**	**Observed size pb**	**Observed heterozigosity**
D22S1045	Tri	TAA	Crom 22, 35.78 Mb	76 109	0,784
D2S441	Tetra	TCTA	Crom 6, 68.21 Mb	78 – 110	0,774
D10S1248	Tetra	GGAA	Crom 10, 130.57 Mb	83 - 123	0,792

NEW AUTOSOMAL MINISTRS STRATEGIES

Different strategies for the development of a new generation of STRs have been proposed (Gill et al. 2006a). Two different but parallel strategies have been adopted according to the different requirements within Europe.

The first strategy employs a 13 STR loci-multiplex that includes three mini-STRs: D12S1248, D22S1045, and D2S441. Laboratories that prefer this strategy will typically wish to work with a single multiplex for all evidential types and will routinely analyse a significant proportion of highly degraded samples. The intention here is that the new multiplex will quickly replace the current multiplex.

On the other hand, the second strategy is to modify a multiplex of six high molecular weight STRs (commonly used), to create smaller amplicons. This will be combined with an additional two loci of high discriminating power, D12S391 and D1S1656. Additionally, it has been proposed to reduce TPOX size. In this case, this new multiplex will be used in conjunction with existing multiplex systems rather than acting as a replacement

These different approaches arise mainly from differences in the emphasis of the type of evidential material that is routinely analysed and in laboratory practices. However, there is full agreement that the overriding requirement is that both strategies must converge to achieve the aim of increasing the number of universal loci to be used throughout Europe, through the use of a new multiplex of 15 STR loci of international level (Gill et al. 2006a).

These new miniSTRs should not replace the actual STRs collection, but complement it, in particular in disaster cases where highly degraded DNA exists (Gill et al. 2004).

Butler (2002) suggested the development by re-engineering of a miniplex that included the 13 CODIS STRs. This system -named "autoplex"-, should have lower allelic ranges, a reduction of "stutters", should cover all 22 autosomal chromosomes and sexual chromosomes, and should exhibit more robust characteristics.

OTHER MINISTRS MULTIPLEXES

Until recently, most studies focused on developing autosomal miniSTRs multiplexes to improve recovery rates from difficult samples. Nevertheless, new efforts have been made to develop alternative procedures for the analysis of degraded DNA, with the successful characterization of Y-chromosome and X-chromosome miniSTRs.

Forensic interest in gonosomal polymorphisms (Y and X chromosomes) has increased notably in the last years, especially Y chromosome studies (Roewer et al, 2001). The singular features of this chromosome, which include haploidy, paternal inheritance and lack of recombination through most of its length, make the Y chromosome a powerful tool for forensic, genealogical and evolutionary studies (Jobling et al. 1997, de Knijff et al. 1997; González-Andrade et al, 2009a). A high amount of Y-STRs and population data have been published (Redd et al. 2002, Hanson et al. 2006, Roewer et al. 2001; González-Andrade et al, 2009b; Martínez-Jarreta et al, 2005). Although there are some commercial kits such as AmpflSTR Y-filer kit (Applied Biosystems) and Power Plex Y sytem (Promega) for routine casework, they are not optimized for working with degraded DNA samples. This is especially accentuated in STR loci exceeding 200 bp, where the possibility of allelic dropout is high,

due to DNA fragmentation. For this reason and with the aim of increasing the success rate of Y-STR typing for degraded DNA, several miniY-chromosomal STR multiplex have been developed.

One of the first studies on Y-chromosome miniSTRs was carried out by Park et al. (2007), creating two Y-miniplexes of 21 reduced Y-STR loci. They included the 17 Y-STR loci of the commercial kit AmpFlSTR® Yfiler™ (DYS19, DYS385, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and GATA H4.1) and other four loci (DYS388, DYS446, DYS447, and DYS449) to augment the discrimination capacity. At the end, they were able to reduce the size of 9 Y-STRs (DYS385, DYS390, DYS391, DYS392, DYS438, DYS439, DYS448, and DYS635); the other primers could not be redesigned because of the prior small size of the amplicon size or other motifs. According to the study, the Y-miniplexes will increase the number of analyzed loci, increasing the discrimination power of the Y-STR profiles. However, they need to be fully forensically validated, requiring a primer concordance study and an evaluation of non-human samples.

Other research group headed by Asamura (2007) has also developed their own Y-miniplexes, concretely two quadruplex systems, which include DYS504, DYS508, DYS522, DYS540, DYS556, DYS570, DYS576 and DYS632. As a result, they obtained PCR amplification products for the eight loci ranged in length from 95 bp (DYS632) to 147 bp (DYS570). Their miniplexes proved to be helpful tools for forensic analysis of degraded DNA samples. Afterwards, the same working group designed new multiplexes for 16 polymorphic Y-STR loci: DYS441, DYS446, DYS462, DYS481, DYS485, DYS495, DYS505, DYS510, DYS511, DYS549, DYS575, DYS578, DYS593, DYS618, DYS638, and DYS643 (products from 91 bp to 151 bp). These new miniplexes were more efficient than the kit AmpflSTR Yfiler when typing degraded DNA (Asamura et al. 2008).

Regarding to the X-chromosome, so far, it has played a minor role in forensic science. Nevertheless, it can be efficiently used to complement the analysis of other genetic markers (autosomal, Y-chromosomal and mtDNA) in forensic casework. It is a very potent tool in complex kinship and deficiency paternity, when the disputed child is female; and in forensic casework to identify the female DNA profile in mixture analysis (Szibor et al. 2003). Although many X-STRs have been validated for forensic testing, additional population studies are needed (Bini 2005, Pereira 2007). As well as in autosomal and Y chromosome STRs, shorter amplicons are necessary to efficiently analyze degraded DNA. Asamura et al. (2006) have designed an effective system for analyzing X-chromosomal short tandem repeats in highly degraded DNA. They generated two miniX-multiplex PCR systems for DXS7423, DXS6789, DXS101, GATA31E08, DXS8378, DXS7133, DXS7424, and GATA165B12. This new miniplexes showed a high effectiveness in analyzing degraded DNA.

Another common problem with degraded DNA samples is to reliably determine their gender. The amelogin test is routinely used to define the sex of DNA samples in forensic cases as it is integrated in most commercial kits. The amelogin gene has two homologous alleles, one on the X chromosome and the other on the Y chromosome, AMELX and AMELY, respectively. Since females have two X-chromosomes, in the typing there will be just one peak corresponding to the AMELX, whereas males (XY) will also have an AMELY peak. However, it has been reported that there can be misleading results, because of primer binding mutations or large-scale deletions in Y chromosome. As a result, males with deletions in the amelogenin gene on the Y chromosome can be erroneously typed as females. To solve

this problem, a new PCR multiplex has been designed by Esteve et al. (2008). It includes 4 mini-X-STR loci and fragments of SRY and amelogenine genes, with product sizes less than 140bp. This short size will be positive to detect the gender when working with degraded DNA.

FUTURE

It is likely that miniSTRs will play a role in the future of degraded DNA analysis probably helping to recover information that would be lost with larger loci from conventional megaplex amplification. Nevertheless, new miniSTRs should not replace the actual STRs collection, but complement it. Furthermore it would be interesting to cover the same loci with STR and miniSTRs primers, so they could be used in routine or difficult cases, respectively. Reasonably, concordance studies would be needed to make possible the comparison of results from both typing methods.

At present, new markers with smaller allele ranges, low stutter and improved characteristics are being studied as possible miniSTRs candidates, which could increase the chance of success in highly degraded samples and the resulting power of discrimination of existing multiplexes.

COMPETING INTERESTS

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

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