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Scope of X-Chromosomal MiniSTRs: Current Developments

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Abstract

The experience of using autosomal short-length amplicon STRs or miniSTRs in profiling of degraded DNA and mass disaster victims is extended into the realm of X-chromosomal (ChrX) STR miniaturization. About half of the total X-STRs are now short-length amplicons and the focus is shifting to using the mini versions of all of them. Joint multiplexing of these loci can be used for solving complex paternity cases and association of mass disaster victims with their families. This technology may herald a new dimension for research in population genetics and evolution. We present an overview of the progress made thus far and the future scope and prospects for X-miniSTRs.

Keywords: Degraded DNA analysis; Mini-STRs; X-chromosomal STRs

Introduction

Sherlock Holmes said, "I had come to an entirely erroneous conclusion which shows how dangerous it always is to reason from insufficient data" [1]. A century later, forensic science has moved forward a long way and instead of crime psychology, it is the empirical evidence that is of interest to a forensic scientist. But now it is even more necessary to have sufficient data for accurate analysis in a forensic laboratory lest the faulty evidence be responsible for the conviction of an innocent citizen [2,3]. As forensic science and its techniques advance, so do the techniques of the perpetrators of the crimes. Moreover, environmental pollution further confounds the evidence found at a crime scene or samples left on a mass disaster site. Hence, there is a constant pressure to develop novel and precise methods to identify the guilty but to exonerate the innocent or to accurately identify a missing relative. From fingerprinting to DNA profiling, many techniques were standardized and discarded until the data basing of core Short Tandem Repeats (STR) loci. Since then, millions of profiles have been generated and now STRs are the workhorses for forensic testing [4].

In forensic casework we are often faced with samples that are not in the best of conditions and consist of DNA that is highly fragmented. This includes burnt items, bones and teeth. Exposure to heat and humidity, aids in the breaking down of DNA molecules to smaller fragments. Nucleases from within the cell attack the DNA as soon as the cell dies, leading to its degradation [5]. Biochemical, microbial or oxidative process can also lead to DNA degradation. In favourable environment DNA is rendered highly fragmented by microbes. Bacteria are the main agents on land while fungi are responsible for oceanic degradation [6,7]. Slightly degraded samples can be typed by traditional STRs, but may yield negative results as the fragmentation increases [8-10]. Conventional STRs have a size range of 100-400 bp most of which consists of flanking sequences on both sides of the repeat region. To alleviate the problems associated with analyzing DNA from degraded samples a new set of STR primers known as Miniplexes were designed by moving the primers closer to the repeat region leaving the extra sequences out [11,12]. Using shorter amplicons in polymerase chain reaction (PCR), improvement has been reported in obtaining results from forensic evidence or a mass disaster site having degraded specimens [13].

The DNA typing has played a pivotal role to establish the paternity of child which is utmost priority for support, inheritance right and other social benefits of a child. Short Tandem Repeats (STRs) located on X chromosome are powerful marker for complex kinship testing such as deficiency paternity testing when the disputed child is a female [14-16].

X-STRs are currently used in parentage analysis and relationship investigations such as avuncular and first cousin relationships. In addition to X-STRs, stable haplotype of closely associated X-chromosome markers have proven to be a powerful tool in kinship analysis especially for cases when father/daughter relationships are to be tested. X-STRs have also advantage over autosomal STRs for paternity cases involving close blood relatives as alternative putative fathers and in deficiency paternity cases, i.e. when the DNA sample from putative father is not available and DNA from paternal relative has to be analyzed instead [17]. Further, X-linked STRs can be used to solve sibship status, without using father's DNA, of two females having the same biological father [18,19]. X-STRs can determine the relationship of grandmother/granddaughter as granddaughter theoretically has to carry at least one allele in common with the grandmother [20]. In forensic analysis of mixed stains, X-STRs are helpful to identify the female DNA [21,22].

History and Progression

The world first came to know about short amplicon STR in 1994s when the British Forensic Science Service (FSS), while running degraded DNA samples from the remains of victims of the Branch Davidian fire in Waco, Texas, found that smaller STRs in their 4-plex work better than the larger loci [23] which was subsequently

corroborated by Clayton et al. [24]. Wiegand et al. (2001) described that by redesigning the primers, the length of the amplicons can be reduced [25]. However, the real work started when the World Trade Centre Kinship and Data Analysis Panel (WTC KADAP), was formed to identify victims of the WTC attacks using DNA testing and the name "miniSTR" was coined. In 2004, a collaborative study between nine European and US laboratories was organised under the auspices of the European DNA Profiling group (EDNAP) on the same sets of degraded samples. The results were collated and analysed and, in general, mini-STR systems were shown to be the most effective [26]. Since then, a number of other studies have demonstrated that successful analysis of degraded DNA specimens from mass disasters or compromised forensic evidence improves with smaller-sized PCR products [8,10,12,14,27-31]. The TIMELINE (Figure 1) shows the progress of ChrX miniSTRs over the years [32-46].

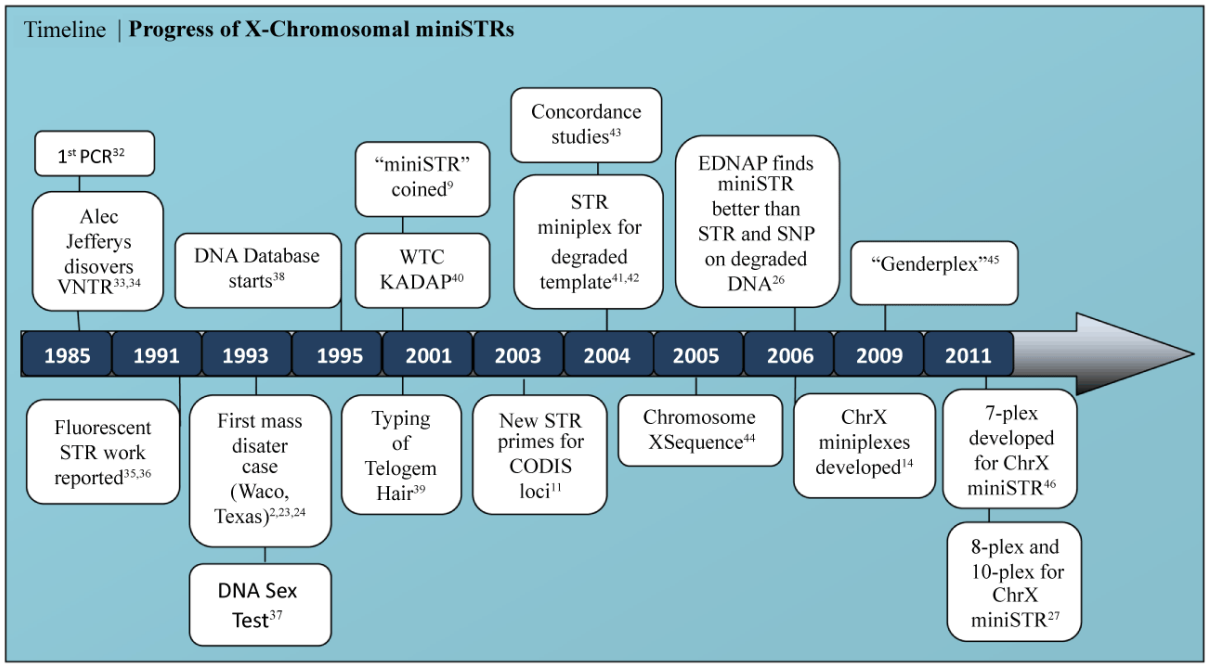

Figure 1: Evolution of miniSTRs – a Timeline. PCR=Polymerase Chain Reaction, VNTR=Variable Number Tandem Repeats, STR = Short Tandem Repeats, WTC KADAP=World Trade Centre Kinship and DNA Analysis Panel, CODIS=COmbined DNA Indexing System, EDNAP=European DNA Profiling group, ChrX=X-Chromosome.

X-Chromosomal STRs and miniSTRs: Identity Testing and Beyond

Due to its unique inheritance pattern, the X chromosome is a potential candidate for forensic and human identity testing applications. Normal males possess one X chromosome and one Y chromosome, whereas females possess two X chromosomes. Now, more than 40 X-STRs have been established as forensic markers [47-50]. The majority of X-STRs can be used routinely and there are no peculiarities in terms of their usage except HumARA, one of the established STR markers. It has been recommended recently that HumARA should not be used as a forensic marker [51] due to its association with bulbar muscular atrophy [52]. X-STR haplotyping can be of particular help in kinship testing with deficient paternity cases where a DNA sample from one of the parents is not available for testing. For example, if a father/daughter parentage relationship is in question, X-STRs may be helpful [53-57].

Usually ChrX markers are less powerful in stain analyses than autosomal markers and are not suitable for use in the testing of male traces where there is female contamination. However, these are more powerful than autosomal markers for identification of female traces in male contamination [58].

The ideal technique for forensic DNA typing is multiplex PCR because as the number of polymorphic loci examined increases, the probability of identical alleles, being present in two different individuals, decreases [59]. Multiplex systems have been developed to use the X-STRs efficiently for paternity testing. The first multiplex study with respect to X-STRs analysed nine loci in three different multiplexes which included duplex PCR (DXS6789 and DXS6795), Triplex PCR (DXS7133, DXS9895 and DXS9898) and Quadruplex PCR (DXS6803, DXS8378, GATA164A09, and DXS7132) [60]. In a very short period of time, other studies came up with parallel amplification of three [61], four [62,60], five [63,64], six [65], seven [66], eight [67] ten [15], eleven [68], twelve [69], and thirteen [70,71], X-STR markers. Multiplexes with a greater number of markers are being developed to obtain a higher degree of discrimination.

However, work on ChrX miniSTRs is very slow as compared to autosomal miniSTRs. As reported in the literature, the amplicon size of a miniSTR is below 200bp, above which chances of getting a complete profile from a degraded sample diminish significantly [42]. Others have suggested that the optimum product size is below 150bp [26]. Only a handful of studies are available on ChrX miniSTRs which are often combined with other larger-amplicon size markers, hence making them unsuitable for use in degraded sample studies and

casework. These markers along with amplicon size range are given in Table 1.

In the table, STRs namely, DXS10147, DXS10160, DXS10162, DXS9902 and DXS10103 are barely under the threshold level for "mini" and hence not suitable for inclusion in a miniplex.

Marker	Size (in base pairs)	Reference
AMELX, AMELY	X=55, Y=58	[45]
DXS7424	79–100	[14]
AMELX, AMELY	X=80, Y=83	[72]
GATA165B12	90–110	[14]
DXS6795	90–111	[60]
GATA31E08	101–133	[14]
DXS8378	95–111	[14]
DXS7423	99–115	[14]
AMELX, AMELY	X=106, Y=112	[72]
DXS7133	106-130	[73]
GATA172D05	108-136	[73]
DXS6803	109-128	[74]
DXS7130	109-128	[74]
DXS6801	113-137	[75]
DXS10163	121–171	[76]
DXS6789	122–162	[14]
DXS7132	131–155	[73]
DXS10160	135 – 193	[77]
DXS9895	139-161	[73]
DXS101	142–169	[14]
HPRTB	144-176	[22]
DXS10165	145–173	[76]
DXS7424	147-174	[78]
DXS10162	150–186	[76]
DXS10159	154–190	[76]
DXS10103	160-200	[79]
DXS9902	162-186	[27]
DXS10147	165–185	[80]

Table 1: MiniSTRs of X-Chromosome along with their product size and references.

The first mini-only multiplex for ChrX STRs was reported by Asamura and colleagues [14]. Designing new primers, they devised two 4-plexes consisting of X-STR loci, DXS7424, DXS101, DXS7423, DXS6789, DXS8378, DXS7133, GATA165B12 and GATA31E08. The miniplex strategy was shown to be effective for the analysis of degraded DNA based on the results of tests with these multiplexes. It was concluded that for personal identification, these multiplex systems offered high effectiveness with degraded DNA samples [14]. However, the problem in their approach is that for any meaningful results, a large quantity of sample is required to perform analysis with so many miniplexes, thus defeating the very purpose of miniSTRs. Samples from a mass disaster site or from an environmentally exposed crime scene may not be only highly degraded but also in very scarce quantity making it difficult for the scientist to perform multiple PCR analyses [53]. In 2011, a 7-plex was developed [46] while an 8-plex and 10-plex were reported elsewhere [27].

In the criminal justice system, it is not uncommon that, due to circumstantial evidence, a person is indicted and sent to jail but years later proved to be innocent through the use of DNA typing [81]. Since evidence is not always found in a very 'DNA-friendly' environment, degradation is one of the most common hindrances in such cases. MiniSTRs have the ability to provide reliable profiles from old samples [82] and, hence, may be used to set the innocent free.

There are several measures like variation in allele size and frequency, heterozygosity, genetic variability and discontinuous allele distribution, which are useful in population genetics. These miniSTRs may be powerful in the study of population genetics and can be used to detect changes due to mutations or genetic drift among populations [83]. Being lineage markers, these can also be used as ancestry informative markers to deduce individual ancestry information from admixture populations [84]. The X chromosome, along with Y-chromosome and mitochondrial DNA, has tremendous potential for the reconstruction of phylogenetic trees to unravel the complexities of the history of populations. But we need many loci for this kind of work and with the help of miniaturization of X-STRs, we would be better equipped to tackle this problem [85].

Another avenue where miniSTRs are potential candidates for use is the study of ancient samples such as found inside and around the archaeological digs. Since the DNA found in sites like these is expected to be highly degraded, only short DNA templates of up to 300bp are present [86] making it challenging for any meaningful analysis with traditional STRs. These 'minis' can prove essential where other anthropological methods cannot be applied for sex typing, phylogenies [87] and to explore the gender differences in the past populations [88, 89]. Although SNPs are potential candidates for use with degraded samples, problems like low polymorphism and difficulty in mixture interpretation hinders their widespread use, outside the research setting [90].

Miniaturization: Problems and Prospects

There has been substantial progress with shortening the length of many ChrX STRs amplicons during the last decade. But there are significant problems which hinders the process of miniaturization. Foremost among these is that, due to size limitation, not many STRs can be fit together in a single multiplex and most of the time only one marker or two markers per dye are run to avoid overlap. This approach normally makes a maximum of 5-10 miniSTRs in a single multiplex using the 6-dye chemistry.

Secondly, most human identity testing applications require a high degree of polymorphism and, hence, a wider allele frequency distributions. In multiplexes, these markers consume precious electrophoretic space. However, in some parentage testing situations,

less polymorphic loci having lower mutation rates are more useful. For example, mutation rates are not significant when evidence is compared directly with a suspect [91]. But in parentage and kinship testing where comparison between relatives is done, as for identification of mass disaster victims, mutational events become important [92]. STRs having long homogenous repeat structures are prone to instability such as DXS8377 and DXS10011 [49] and are, therefore, not suitable for use [93]. Hence, STR loci possessing a smaller size range and sufficiently polymorphic are being characterized [12].

Thirdly, miniSTR primers anneal to different locations than those of the conventional STR primers, and polymorphic nucleotides or insertion/deletions may be present in the flanking regions surrounding the STR repeats. Discordant results may be produced due to allele dropout and size shifts in amplicons generated from one of the primer sets [94,95].

Fourthly, there are analytical issues attached to the miniaturization approach. When the size is reduced to less than 150bp, the biggest problem comes from dye blobs. These may be left over from the oligonucleotide synthesis process or may result from dyes falling off the primer during the heating and cooling steps of PCR [76].

Lastly, smaller PCR products have a problem in the manner in which sizing is performed using an internal lane standard. Local Southern, the default method used by the GeneScan software, requires two peaks from the internal size standard to be present on either side of a peak being defined. Hence, both 35 and 50bp peaks from the GS500 Liz size standard must be defined for measuring any allele below 75 bp [11]. Due to the presence of primer dimers in the smaller sizing area, sizing peaks may become ambiguous. Therefore, it is recommended that Global Southern, where a regression line generated from all sizing peaks is used to fit the STR alleles, be the method of choice for small-size amplicons [96].

Conclusion

X-chromosomal miniSTRs offer a new potential tool for recovering useful information from samples that are otherwise difficult to generate full profiles. These miniplexes are valuable in the analysis of samples where allele dropout and reduced sensitivity of larger STR alleles occur. They are also useful for improving the power of exclusion in mass disaster cases where there are insufficient family references for association. In complex paternity cases, these loci can provide additional discrimination in parentage analysis. It should be kept in mind that the purpose of these miniSTRs is not to replace but to supplement the current battery of autosomal core loci where samples are degraded or there is not enough reference material available for identification profiles. It would prove useful if future loci for consideration in forensic casework applications contain a more compact allele range and be able to be amplified as small PCR products.

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