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Characterization and performance of new MiniSTR loci for typing degraded samples

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Abstract. Forensic DNA analysts often perform short tandem repeat (STR) typing on highly degraded biological material and then turn to mitochondrial DNA testing if many or all of the STRs fail. The commercially available kits for multiplex amplification of the 13 CODIS (FBI's COmbined DNA Index System) STR loci usually exhibit allele or locus-dropout for larger sized loci with degraded DNA or samples containing PCR inhibitors. By moving PCR primers closer to the STR repeat region, we have demonstrated that it is possible to obtain fully concordant results to the commercial kits while improving successful analysis of degraded DNA with smaller PCR products or "miniSTRs." However, many of the CODIS core loci have large allele ranges (e.g., D21S11 and FGA) that make it impossible to create small PCR products. We have examined a battery of new candidate STR loci that can be made less than 100 bp in size (in many cases), and would therefore be helpful in testing highly degraded DNA samples. A set of six non-CODIS markers has been characterized and published, and the performance of these markers on degraded materials such as aged bloodstains and shed hairs has been examined. Here we report on the status of 10 additional miniSTR markers unlinked from the CODIS STR markers.

Keywords: MiniSTR; Degraded DNA; Short tandem repeat; Forensic DNA typing; D10S1248; D14S1434; D22S1045

1. Introduction

The forensic DNA typing of nuclear STR loci using a common set of core genetic markers (such as the 13 CODIS markers) has played an important role in criminal investigations and cases involving forensic identification, for example the association of remains from mass disasters. Oftentimes in mass disaster investigations, the forensic

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scientist must analyze biological material that has been highly degraded from exposure to the elements, or has been contaminated with PCR inhibitors. Frequently, the commercially available kits for multiplex amplification of the CODIS STR loci will often fail to amplify the larger sized PCR products, resulting in allele or locus-dropout with degraded DNA or samples containing PCR inhibitors. In highly degraded samples, the forensic scientist may rely upon mitochondrial DNA typing to obtain a result.

A number of studies have demonstrated that successful analysis of degraded DNA specimens from mass disasters or forensic evidence improves with smaller sized polymerase chain reaction (PCR) products, also referred to as "miniSTRs" [1]. One advantage of developing miniSTRs for the CODIS loci is that it is possible to obtain fully concordant results compared to the commercial kits while improving the successful analysis of degraded DNA [2]. However, many of the CODIS core loci have large allele ranges (e.g., D21S11 and FGA) or flanking sequences adjacent to the repeat motif (e.g., D7S820) that makes it impossible to create small PCR amplicons [1].

2. Non-CODIS miniSTRs

We have developed a set of novel STR markers that are not genetically linked to the CODIS loci for analyzing degraded DNA samples. We have examined a battery of STR markers that have a narrow allele range (e.g., less than 50 bp), have heterozygosity values of 0.70 or greater, and have "clean" flanking sequences that can generate PCR amplicons ~100 bp [3]. Two triplex non-CODIS (NC) markers have been characterized and published [3]. The NC01 miniplex (D10S1248, D14S1434, and D22S1045) performed well in an EDNAP/ENFSI collaborative study on degraded DNA analysis [4]. These markers have recently been recommended for inclusion in the next generation STR multiplex kit for Europe [5].

3. Results

Fig. 1 represents the chromosomal map of the human genome. Mapped upon the chromosomes are the 13 CODIS markers and the 6 previously described NC markers [3]. Recently, we have

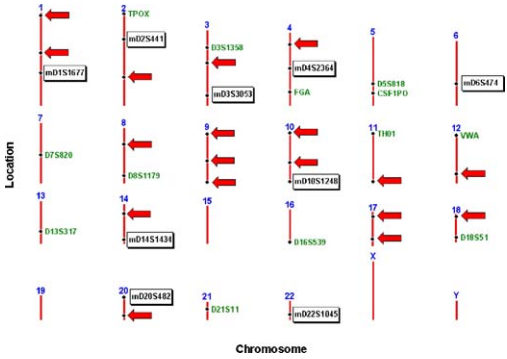

Fig. 1. Chromosome map with the CODIS and non-CODIS markers. The 13 CODIS markers are labelled without boxes. The published NC markers [3] and the NC03 markers are denoted by boxes. The remaining 18 markers under investigation are indicated by arrows. The lower case "m" before each marker refers to "mini".

Table 1
Candidate markers for novel miniSTRs

Candidate marker	Repeat type	Observed allele range	Allele size range (bp)	Caucasian ($n=262$)	African American ($n=260$)	Hispanic ($n=140$)
D3S3053	Tetra	8–14	84–108	0.724	0.713	0.814
D6S474	Tetra	11–18	107–135	0.802	0.765	0.679
D20S482	Tetra	9–19	86–126	0.689	0.673	0.729
D1Sa	Tetra	7–13	81–105	0.632	0.673	0.727
D1Sb	Tri	10–16	81–99	0.737	0.783	0.693
D2Sa	Tetra	6–14	127–161	0.801	0.740	0.734
D3Sa	Tetra	12–19	111–109	0.723	0.752	0.829
D4Sa	Tetra	7–13	85–109	0.709	0.752	0.691
D9Sa	Tetra	9–17	93–125	0.742	0.753	0.686
D12Sa	Tri	10–20	76–106	0.842	0.788	0.879

The first three markers have been selected as the NC03 miniplex. Repeat type, the observed number of alleles, the allele size range, and observed heterozygosity values for U.S. Caucasian, African American, and Hispanic populations are shown.

developed 21 new potential miniSTR markers unlinked from the CODIS loci. Ten of these markers have been characterized and over 600 population samples from U.S. Caucasians, African Americans, and Hispanics have been genotyped (Table 1). Of the 21 potential markers, three have been developed into the next NC triplex, NC03 (D3S3053, D6S474, and D20S482).

4. Conclusions

These miniplex sets should prove valuable in the analysis of samples where allele dropout and reduced sensitivity of larger STR alleles occur or can be useful for improving the power of exclusion in cases where there are insufficient family references for association in mass disasters. These loci can also provide additional discrimination in parentage analysis, especially in complex paternity cases (e.g., incest) where closely related individuals share multiple alleles. Additional miniplexes will be published along with primer sequence information and population statistics and will be made available at the STRBase website: http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm.

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