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Development and characterization of two mini-X chromosomal short tandem repeat multiplexes

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ABSTRACT

This study presents the development and characterization of two X chromosomal short tandem repeat (STR) multiplexes utilizing reduced-size amplicons (less than 200 base pairs) for identity and kinship testing with degraded DNA. Approximately 1360 samples across 4 U.S. population groups were typed for 15 X chromosomal STR markers: DXS6789, DXS7130, DXS9902, GATA31E08, DXS7424, GATA165B12, DXS101, DXS6795, GATA172D05, DXS10147, DXS8378, DXS7132, DXS6803, HPRTB, and DXS7423. A high degree of polymorphism was observed for each marker and both multiplexes were sensitive down to 200 pg of pristine DNA. The two proposed multiplexes are suitable for forensic use, and show potential for improved analysis of compromised bone samples.

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1. Introduction

The multiplex detection and analysis of short tandem repeat (STR) markers is a common tool used for genetic identity testing in the forensic setting. Numerous publications have characterized genetic markers located throughout the autosomes and male-specific Y chromosome that can be used for this purpose. More recently, markers located on the X chromosome have emerged as additional tools in this forensic arsenal. X chromosomal STRs can be used to supplement traditional kinship testing due to their unique inheritance pattern and, correspondingly, the breadth of published literature on the subject has expanded greatly in recent years.

For both the Y chromosome and the autosomes, commercial kits are available that probe a wide variety of genetic markers (see Refs. [1–5], for example). When it comes to the X chromosome, however, only one kit is currently being manufactured with limited availability, the Investigator Argus X12 kit (Qiagen, Hilden, Germany). This kit simultaneously amplifies and detects twelve X chromosomal STRs (DXS8378, HPRTB, DXS7423, DXS7132, DXS10134, DXS10074, DXS10101, DXS10103, DXS10148, DXS10146, DXS10079, and DXS10135) plus amelogenin in four fluorescent dye channels [6]. With amplicon sizes ranging from approximately 104 to 375 base pairs (bp), the kit's detection limit is 100 pg of input DNA [6]. Noncommercial multiplex assays have also been published, amplifying 2–12 loci in a single reaction (see Refs. [7–19], for example).

At the Armed Forces DNA Identification Laboratory (AFDIL), kinship testing is routinely used to support the identification of skeletal remains. Many times, mitochondrial DNA (mtDNA) typing will reveal the answer to the question of identity, particularly in closed populations and when a direct maternal reference is available. However, where maternal references are unavailable or where the unidentified individual matches one of the most common mtDNA haplotypes, mtDNA testing alone may be inadequate. Sufficient statistical power must then result from fewer, smaller-amplicon STR loci or low copy number analyses. In such cases, markers on the X chromosome may provide additional information. Consequently, the selection of candidate X chromosomal markers and the development of these markers into STR multiplexes with reduced amplicon sizes (or "mini-STRs") offers the potential to augment both traditional STR testing and mtDNA sequencing. Other applications of X chromosomal STRs include immigration or maternity cases as well as paternity cases with female children. X chromosomal markers may also prove useful as a complement to mtDNA and Y chromosomal markers in the study of human evolutionary history [20].

In general, STR markers are selected for forensic use based upon their observed heterozygosity, discriminatory power, and repeat size/structure. Another criterion for selecting candidate STR markers

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is pairwise linkage; in order to take advantage of the product rule in the calculation of random match statistics, markers should not be linked to one another. Marker selection restricted to a single chromosome introduces an additional challenge because some loci will necessarily be linked. Additionally, because DNA templates encountered in the forensic setting are often degraded (for example, as the result of prolonged exposure to environmental extremes), amplicon size should be considered in selecting potential markers. In such cases, shorter amplicon sizes are favored with the goal of recovering the maximum number of alleles [21]. In a study examining degraded samples, Asamura et al. demonstrated the success of two X chromosomal STR quadruplex reactions consisting of amplicons ranging from 76 to 169 bp in length [13].

Here, two complementary mini-X chromosomal STR multiplexes, one 8-plex and one 10-plex, were assembled with the above parameters in mind. Markers were selected to maintain high heterozygosity and represent all four X chromosomal linkage groups identified thus far [22,23]. Further, all amplicon sizes were kept small (<190 bp) to facilitate application to degraded samples. This study describes the development and optimization of these two multiplexes as well as their application to the analysis of four U.S. population groups: African Americans, U.S. Asians, U.S. Caucasians, and U.S. Hispanics.

2. Materials and methods

2.1. Selection of markers and primer design

A review of X chromosomal STR literature resulted in a list of potential markers. Forensic utility was assessed according to the following criteria: (a) potential for small amplicon size; (b) heterozygosity in published materials; and (c) distribution between the proposed linkage groups across the X chromosome. Markers best matching these criteria (Table 1) were chosen for inclusion into two multiplexes and organized according to amplicon size (Table 2).

Approximately 200 bp flanking either side of the repeat regions for the chosen markers were downloaded from the UCSC Genome Browser [24] using their BLAT In Silico PCR search [25] and the published primers. In many cases, published amplification primers were sufficient for incorporation into the multiplexes, but several markers required one or both primers be redesigned (see Section 3.1). When necessary, primers were designed using the web-based program Primer3 [26], and selected primers were screened for use in multiplex reactions using the web-based algorithm AutoDimer [27].

One primer for each marker was labeled at the 5′ end with a fluorescent dye, either 6FAM, VIC, NED, or PET (Applied Biosystems, Foster City, CA). A tail was added to the complementary primer in the set at the 5′ end in order to promote the complete adenylation of PCR products [28] and, in some cases, provide adequate spacing between amplicons in the multiplex. This tail was either GTTTCTT, ATT, or a single G (Table 2).

2.2. Source and extraction of DNA samples

Unrelated, anonymous bloodstains represented four U.S. populations: African Americans (174 females, 175 males), U.S. Asians (300 females, 201 males), U.S. Caucasians (146 females, 122 males), and U.S. Hispanics (122 females, 123 males). Bloodstain cards were extracted on the Biomek® 2000 robot (Beckman Coulter, Brea, CA) using the DNA IQ™ system (Promega Corporation, Madison, WI) or on the Qiagen 9604 robot using the Qiagen QIAmp DNA kit (Qiagen, Gaithersburg, MD). Three control DNA standards – 9948 (Applied Biosystems and Promega Corporation), 9947a (Applied Biosystems), K562 (Promega Corporation) – were also characterized, as per the recommendations of Szibor et al. [29]. Results were compared to published profiles before allele designations were made (see Section 3.2 and Table 1).

2.3. STR amplification, detection, and analysis

Amplification was performed in a 10 μL reaction that consisted of 1× PCR buffer II (Applied Biosystems), 2 units AmpliTaq Gold™ DNA polymerase (Applied Biosystems), 0.25 mM dNTP Mix (Applied Biosystems), 0.15 mg/mL bovine serum albumin (Sigma–Aldrich, St. Louis, MO), 2 mM magnesium chloride solution (Applied Biosystems) and 2 μL of primer mix. Primer mix concentrations were adjusted empirically to balance peak heights within each multiplex and individual concentrations are listed in Table 2. Thermal cycling was performed on a GeneAmp® 9700 (Applied Biosystems) using the following parameters: initial incubation at 96 °C for 10 min, amplification with 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, extension at 60 °C for 45 min, and final soak at 4 °C.

Samples were prepared for capillary electrophoresis by adding1 μL amplified product to 8.7 μL Hi-Di™ formamide (Applied Biosystems) and 0.3 μL LIZ-500 size standard (Applied Biosystems). Samples were injected at 3.0 kV for 10 s and run using a 36 cm array and POP6 on a 3130xl Genetic Analyzer (Applied Biosystems). Data were analyzed using Genemapper ID version 3.2 or ID-X version 1.1 (Applied Biosystems). As mentioned, allele designations were achieved through comparison with control DNAs 9947a, 9948, and K562. Final bins and panels were created based upon an average of all alleles observed in the profiles of population samples.

Table 1
Characteristics of the 15 X-STRs examined in this study.

Marker name	Observed allele range	Repeat motif	Nomenclature reference	Linkage group[a]	DNA profile 9947a
DXS6789	14–25	(TATC)(0-l)-(TATG)x-(TATC)y	[44]	2	21, 22
DXS7130	9–14, 16, 13.3–18.3	(TATC)5-ATC(0-l)-(TATC)x	[47]	2	15.3, 15.3
GATA31E08	7–16	(AGGG)x-(AGAT)y	[52]	4	13, 13
DXS7424	9–20	TAA	[43]	2	14, 16
GATA165B12	8–13	AGAT	[45]	2	9, 11
DXS101	14–31, 33	(CTT)x-(ATT)y	[50]	2	24, 26
DXS6795	6, 8–17	ATT-ATC(0-l)-(ATT)x	This study	1	12, 13
GATA172D05	6–13	TAGA	[46]	2	10, 10
DXS10147	5–11	AAAC	[49]	4	8, 8
DXS8378	8–15	CTAT	[43]	1	10, 11
DXS7132	10–18, 16.3	(TCTA)x-(TCA)(0-l)-(TCTA)2	[46]	2	12, 12
DXS6803	7–14, 16, 10.3–14.3	(TCTA)x-(TCA)(0-l)-TCTA	[47]	2	11.3, 12
HPRTB	7–16	ATCT	[43,48]	3	14, 14
DXS7423	8, 12–17	(TCCA)3-(N8)(0-l)-(TCCA)x	[46,51]	4	14, 15
DXS9902	7–14, 10.1–12.1	GATA	[43]	1	11, 11

[a] According to Refs. [22,23,33]. N8 = TCTGTCCT.