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Highly multiplexed assays for measuring polymorphisms on the Y-chromosome

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Abstract

A multiplex PCR assay capable of amplifying 20 Y-chromosome short tandem repeat (STR) markers simultaneously has been developed. These markers include all of the Y STRs that make up the "extended haplotype" used in Europe (DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and YCAII) plus additional polymorphic Y STRs (DYS437, DYS438, DYS439, DYS447, DYS448, DYS388, DYS426, GATA A7.1, and GATA H4). Single nucleotide polymorphism (SNP) assays that may be rapidly analyzed with time-of-flight mass spectrometry have also been developed using Y-chromosome bi-allelic markers. With improved multiplexes and techniques for more rapid data collection such as mass spectrometry, Y-chromosome haplotypes may be more quickly generated for DNA databases and population studies and forensic cases may be solved more efficiently.

Keywords: DNA typing; Y-chromosome; Short tandem repeat; Multiplex PCR; Single nucleotide polymorphism

1. Introduction

The Y chromosome has long been recognized as a useful tool in forensic investigations, human identity testing applications, and human migration and evolutionary studies [1–3]. In recent years, a number of polymorphic short tandem repeat loci on the Y chromosome have been discovered [3–5]. However, due to the fact that no recombination occurs along the vast majority of the Y-chromosome, the product rule cannot be used to increase the power of discrimination as when using multiple autosomal markers on separate chromo-

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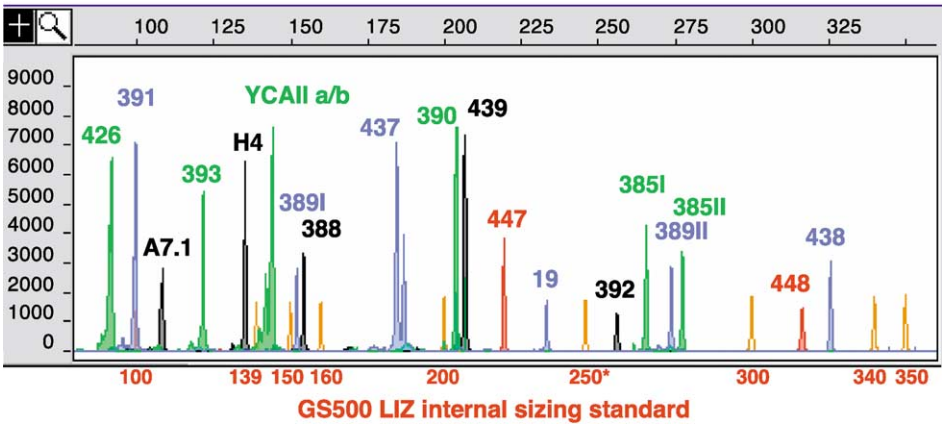

Fig. 1. Result from ABI 3100 Genetic Analyzer viewed in GeneScan® 3.7 using a Y STR multiplex capable of amplifying 20 markers simultaneously. The PCR products are labeled in four different dye colors with a fifth dye (LIZ) used to label an internal-sizing standard.

somes. Thus, the most efficient way to obtain higher levels of discrimination between unrelated samples is to invoke multiplex PCR assays where independent sites that may be widely separated on the Y-chromosome are simultaneously targeted.

As the literature contains references to Y STR multiplexes that at most only amplify five or six loci simultaneously [6,7], we have made an effort to increase the level of multiplexing for Y STRs. We have successfully demonstrated a Y STR 10-plex [8], and we now have a megaplex capable of simultaneously amplifying 20 different Y STR markers (Fig. 1). Our megaplex includes all of the Y STRs that make up the "extended haplotype" used in Europe (DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and YCAII) plus additional polymorphic Y STRs DYS437, DYS438, DYS439, DYS447, DYS448, DYS388, DYS426, GATA A7.1, and GATA H4. We are also developing single nucleotide polymorphism assays from the Y-chromosome that may be rapidly analyzed with time-of-flight mass spectrometry [9,10]. With improved multiplexes and techniques for more rapid data collection such as mass spectrometry, Y-chromosome haplotypes may be more quickly generated for DNA databases and forensic cases may be solved more efficiently.

2. Materials and methods

In order to develop new multiplex Y STR assays, schematic layouts were made to illustrate potential allele size ranges and dye labels for each marker (Fig. 2). The allele size ranges were defined through extensive literature searches and testing of the Y-chromosome consortium (YCC) panel available from Mike Hammer at the University of Arizona. Primers were then designed using Primer 3 [11] with defined PCR product sizes to match the required allele range. Candidate primers were screened for potential primer cross-reactions using a custom designed program written in Visual Basic [12]. Primers were

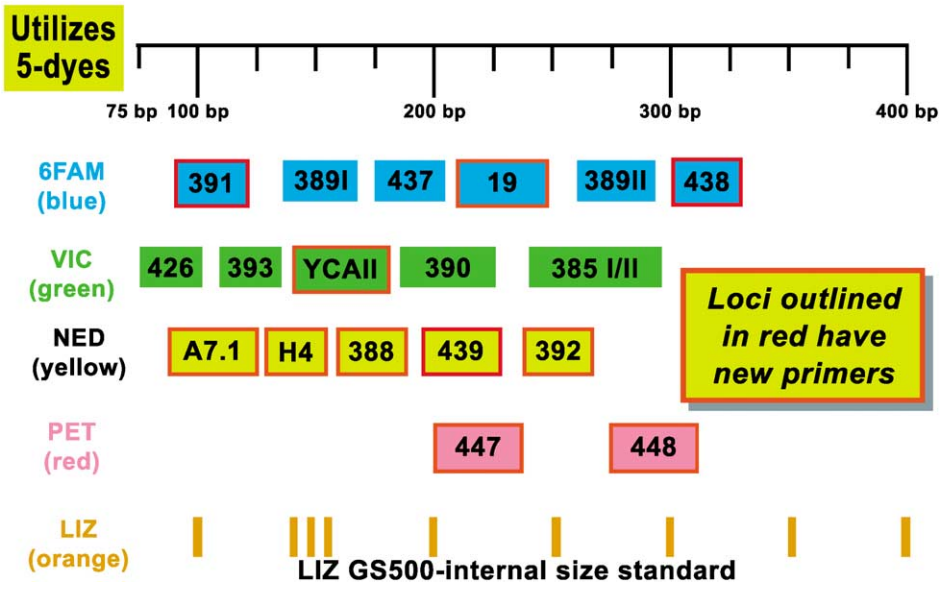

Fig. 2. Schematic of Y STR markers amplified in our 20-plex. Each box indicates the allele size range and dye label for each marker. New primers have been designed for many of the Y STR markers in order to produce compatible allele size ranges.

purchased from MWG Biotech (High Point, NC) or Applied Biosystems (Foster City, CA) and were quality control tested prior to further use to confirm proper synthesis [13].

PCR primer sequences and amplification conditions will be described elsewhere (Schoske, et al., in preparation). Samples were run on both the ABI 310 and 3100 Genetic Analyzers (Applied Biosystems) using manufacturer recommended electrophoresis conditions and appropriate matrix standards available from Applied Biosystems to match the dye sets used.

Multiplex PCR was performed with 5 Y single nucleotide polymorphism (SNP) markers as previously described [12]. Primer extension reactions were then performed as described elsewhere [10] using a Bruker BIFLEX III time-of-flight mass spectrometer (Bremen, Germany) in reflectron mode.

3. Results and discussion

Over 200 publications from the literature were screened in order to determine which Y STR markers were most commonly used and therefore should be included in our megaplex reaction. Other loci that were considered but not included at this time in our megaplex include G09411, A7.2, C4, DYS441, DYS442, DYS446, DYS449, and DYS450. Published primer sequences were used for some of the loci in our Y STR 20-plex but new ones were developed to fit PCR product sizes into the desired size ranges (Fig. 2) or to avoid X-chromosome sequence homology so no amplicons would be generated in female DNA

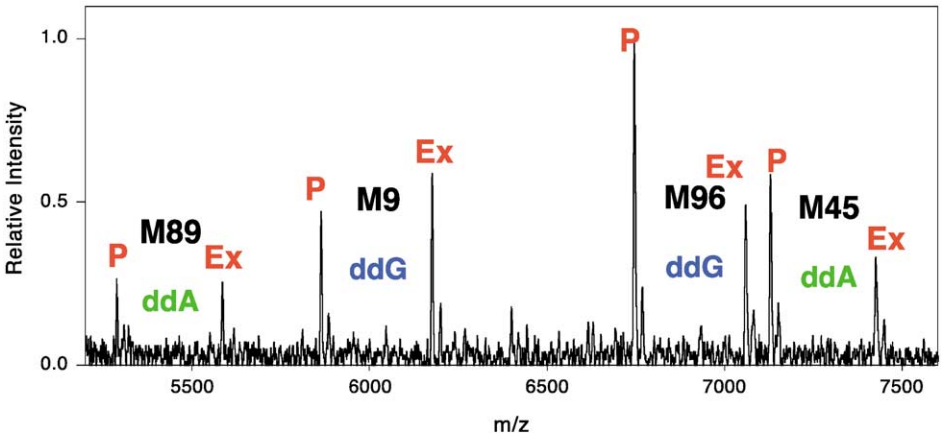

Fig. 3. Y SNP multiplex results using time-of-flight mass spectrometry. The four markers were typed simultaneously in only a few seconds using a multiplex primer extension assay. The mass difference between each primer (P) and the extension product (Ex) identifies the nucleotide present at the probed site for each Y SNP tested.

samples (e.g., DYS391). Preliminary results indicate that this Y STR 20-plex can detect less than 500 pg of male DNA with 28 cycle PCR and is male-specific in DNA mixtures containing in excess of 100 times female DNA. Interlaboratory validation studies with this Y STR 20-plex are underway.

The rapid and accurate capabilities of time-of-flight mass spectrometry are being applied to Y SNPs using multiplex primer extension assays with mass-resolvable markers (Fig. 3). In addition, we have performed male-specific multiplex PCR with as many as 17 different Y SNPs simultaneously [9]. We are also working on a set of well-characterized male DNA samples that can be used as reference materials for laboratories performing Y-chromosome STR and SNP analysis. We hope to make these materials available as NIST Standard Reference Material 2395 sometime in 2002.

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