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Multiplexed assays for evaluation of Y-SNP markers in US populations

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Abstract. Genetic markers located on the Y chromosome are of increasing importance in human identity testing. In an effort to evaluate the forensic utility of Y chromosome single nucleotide polymorphism (SNP) markers, we constructed several novel multiplex allele-specific primer extension (ASPE) assays and utilized a new commercial allele-specific hybridization (ASH) multiplex kit to examine 50 Y-SNP markers in 229 males from two US Caucasian and African American populations. The novel ASPE assays covered 18 Y-SNP markers in three multiplex reactions while a commercial ASH kit was used to type 42 Y-SNPs plus amelogenin for sex-typing purposes. There were 10 overlapping loci between the ASPE and ASH methods permitting an evaluation of concordance on over 2000 allele calls. The 50 Y-SNP markers examined in this study define 45 of the 159 possible Y Chromosome Consortium (YCC) haplogroups. Only 18 different haplogroups were observed in our samples. © 2003 Published by Elsevier B.V.

Keywords: Forensic science; Y chromosome; Single nucleotide polymorphism; SNP typing; Y-SNPs; Primer extension; Allele-specific hybridization

1. Introduction

In recent years, single nucleotide polymorphisms (SNPs) have become more widely used in a variety of applications including medical diagnostics, population genetics, and human identity testing [1,2]. Over 250 single nucleotide polymorphisms (SNPs) have been characterized on the non-recombining region of the Y chromosome [3,4]. Two SNP detection formats capable of multiplex analysis are allele-specific primer extension (ASPE) and allele-specific hybridization (ASH), which we use here to examine variation along the Y chromosome.

2. Materials and methods

2.1. DNA samples

Anonymous liquid blood samples with self-identified ethnicities were purchased from Interstate Blood Bank (Memphis, TN) and Millennium Biotech (Ft. Lauderdale, FL).

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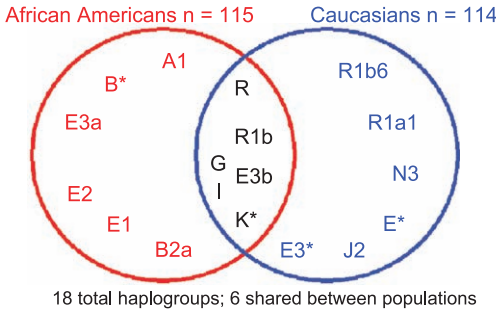

Fig. 1. Summary of YCC haplogroups observed in our sample set.

2.2. Y-SNP markers

The 50 Y-SNPs examined in this study were SRY + 465, $SRY_{10831a,b}$, SRY_{9138}, M130 (RPS4Y), M2, DYS391, M168, M170, M182, Tat, M174, M172, M201, M198, M175, M207, M3, M5, P3, M153, M9, M11, M18, M31, M32, M33, M35, M37, M52, M119, M137, M124, M123, M122, M166, M112, M146, M42, M45, M157, M150, M60, M75, M69, M87, M89, M94, M95, P4, and P25. These loci cover the range of known Y-SNP haplogroups. ASPE typing was conducted with SNaPshot reagents (Applied Biosystems, Foster City, CA) and three in-house 6plex assays [5]. ASH typing was performed with the Signet™ Y-SNP Identification System (Marligen Biosciences, Ijamsville, MD), which enables analysis of 42 Y-SNPs in five different multiplex reactions using xMAP™ suspension array technology on the Luminex® 100 flow cytometer (MiraiBio, Alameda, CA). Testing was performed according to the kit protocols using 2 ng of genomic DNA for each multiplex reaction.

2.3. Allele specific primer extension assays

The extension primers were 5′ -tailed with poly-T sets to enable electrophoretic mobility differences between the various loci examined in the multiplex. Primer sequences, reaction conditions, and electrophoretic parameters for the in-house assays are described elsewhere [5].

3. Results and discussion

The 50 markers examined here define 45 of the 159 possible Y Chromosome Consortium (YCC) haplogroups [4]. We examined 114 US Caucasians and 115 African American samples across these 50 Y-SNPs and found 18 different haplogroups (Fig. 1). It is interesting to note that simple analysis of only two Y-SNPs, M2 and P25, would have accurately reflected over 60% of the 229 samples in these population data sets. Additional information from the other 48 markers typed did not further refine the position of these samples on the YCC haplogroup tree.

We observed full concordance between allele calls made with the primer extension and hybridization methods across more than 2000 allele calls. With the ASH method, allele calls are made based on the highest signal observed when both alleles are examined for an

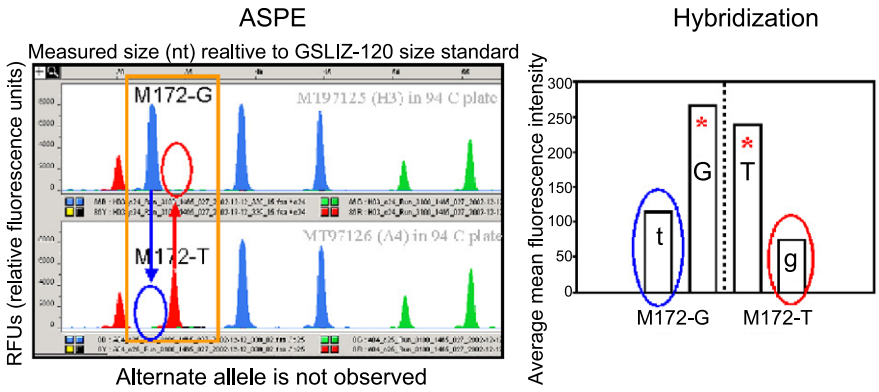

Fig. 2. Comparison of signal-to-noise observed with primer extension vs. hybridization measurements on the same samples with the Y-SNP M172 (G or T). The background signal for the alternate allele is circled. The S/N ratio for the M172 hybridization assay is 2.3 and 3.2 for the G and T alleles, respectively.

SNP marker. The primer extension technique provided a cleaner background than allele-specific hybridization (Fig. 2).

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