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The evaluation of an autosomal SNP 12-plex assay

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Abstract. SNPs have the potential to play a useful role in human identification testing. Small PCR amplicon sizes associated with SNP typing technologies make SNPs attractive for typing degraded DNA or other low copy number situations. SNP markers can be useful in combination with STRs for resolving complex paternity issues (e.g. incest), identifying victims of mass disasters where insufficient family references are available and possibly inferring population of origin. Important considerations for SNP markers are the larger number required to equal the discriminatory power compared to traditional STRs, their inability to resolve complex mixtures, issues related to databasing new loci and the availability of a standard analysis platform.

Keywords: SNP; Autosomal marker; Multiplex PCR; Allele specific primer extension; NIST

1. Introduction

Various SNP typing platforms exist, but at this time there is not a universally accepted platform for SNPs and human identity testing. Currently, we are typing SNPs with multiplex allele specific primer extension (ASPE) reactions. The assay is comprised of an initial step of PCR followed by primer extension and subsequent fragment separation and detection by capillary electrophoresis. ASPE multiplex panels can routinely type 6–12 SNPs in a single tube and have reported to go as high as 35 SNP markers [1–4]. We have recently developed a 12-plex SNP assay that has been used to type over 600 NIST U.S. population samples. The 12 markers are a subset of 70 bi-allelic SNP markers that were previously typed in our laboratory [3]. The amplicon sizes range between 62 and 110 base pairs. These 70 SNP markers were typed for 189 U.S. samples: 74 Caucasians, 71 African Americans and 44 Hispanics. The 70 SNPs were typed using a panel of 6-plex PCR/ASPE (allele specific primer extension) reactions.

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The allele frequency distribution for the 70 loci ranged from 0.25 to 0.74. We selected 12 of the 70 loci to incorporate into a multiplex assay. The 12 loci selected had an observed heterozygosity of >0.45 in each of the three U.S. sample groups examined. The 12 loci lie primarily on non-CODIS loci chromosomes. The assay is being evaluated with a focus on determining the power of discrimination and sensitivity compared to traditional STR markers.

Practical and inherent characteristics of SNP markers will likely prevent them from replacing traditional STR typing methods [5]. However, SNP markers can provide valuable complementary roles in human identity testing. Small autosomal panels of SNPs for typing challenging DNA samples are an example of where SNPs can benefit the forensic community. In order to illustrate an example of a 'challenging sample', we will discuss SNP typing results for an aged blood sample compared to results obtained from a commercial STR typing kit.

2. Materials and methods

2.1. SNP loci

The loci present in the 12-plex assay are listed in Table 1. The chromosomal location of each locus is listed along with the corresponding dbSNP reference number and PCR product size. Three of the loci (1, 6 and 10) are located on chromosomes 11 and 13 that already contain CODIS loci (specifically D13S317 and TH01).

2.2. Multiplex PCR and ASPE assays

The 12-plex PCR and ASPE assays were designed using a similar primer screening strategy described earlier [2,6]. PCR amplification conditions used 32 cycles with an annealing temperature of 55 °C. A total volume of 15 μL was employed with 2 mM Mg^{+}, 1× Taq Gold PCR buffer and 1.5 units of Taq Gold polymerase. The ASPE reaction was performed using the ABI SNaPshot kit reagents. Extension primers incorporated 5′ end 'T' tails for shifting electrophoretic mobility. The fragments were separated on an ABI 3100 with previously described electrophoresis run conditions [2].

Table 1
Candidate SNP loci for the 12-plex assay

Locus	Chromosome	Chr. position	dbSNP reference	PCR product size (base pairs)
1	13	68,098,515	rs2018205	108
2	15	20,547,981	rs999842	64
3	10	82,436,151	rs922992	63
4	1	164,087,184	rs2013526	65
5	17	32,063,659	rs727206	68
6	13	37,899,740	rs730249	65
7	17	79,577,854	rs868432	70
8	1	109,978,841	rs924181	63
9	6	15,118,209	rs927628	70
10	11	131,628,725	rs921269	76
11	20	43,388,976	rs4467339	66
12	15	58,792,647	rs877228	62

3. Results and discussion

3.1. Aged blood stain typed with Identifiler

A blood stain was stored on paper (Whatman 903) at room temperature for 15 years. A portion of the stain was subsequently Chelex extracted and refrigerated for 4 years. The sample was quantified on an ABI 7500 instrument using the commercial real-time PCR Quantifiler kit according to the manufacturer's protocols. Approximately 1 ng of the extract was amplified with the Identifiler STR typing kit following the manufacturer's recommendations. Only five of the loci (D8, D3, D19, D13 and Amelogenin) were typed. Two loci, TH01 and VWA, gave partial typing results.

3.2. Aged blood stain typed with 12-plex SNP assay

A portion of the same DNA extract (1 ng) was amplified with the 12-plex PCR primers. This was followed by the 12-plex primer extension reaction. A full profile was obtained for all 12 SNP loci with signal above 750 RFUs. The random match probability (RMP) was calculated for the 12 locus profile using allele frequency data obtained from running over 600 U.S. samples. The RMP for the aged stain sample was calculated to be 1 in 19,954. The lower molecular weight amplicons were efficiently amplified by the 12-plex assay and exhibited good overall signal balance between loci.

3.3. Summary

We have typed over 1000 pristine DNA samples with the 12-plex assay for databasing purposes. The assay has proven to be robust and able to successfully type 0.5 to 1 ng of input DNA. The RMP was also calculated for the four STR loci; this value was 1 in 139,807 [7]. Assuming that the STRs and SNPs are not linked, combining the information gives a RMP of 1 in 2,789,742,641. This suggests that combining even relatively small panels of SNP loci with a partial STR profile can be useful when examining challenging samples.

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