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Development of a genotyping assay for the UK, European, and CODIS core STR loci identifying length and sequence variation in the target loci

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ABSTRACT

We have developed a highly automated assay covering twenty one markers spanning the UK, European, and CODIS core short tandem repeat (STR) loci. In this assay, sample DNA is amplified with an 8-well PCR panel, and the masses of the PCR products are determined on an electrospray ionization-mass spectrometry platform. The mass measurements are accurate enough to assign a base composition to the PCR products, specifying the number of their constituent dA, dG, dC, and dT residues. The base compositions in turn define the STR genotypes. Sequence variants have been observed in many of the STR loci, and the accuracy of the mass measurements supports their identification with this assay. Parameters in the initial evaluation of the assay included species specificity, sensitivity, reproducibility, accuracy, and concordance. Results indicate that the assay is concordant with existing assays while providing additional information by virtue of the detection of sequence variants of STR alleles.

1. Introduction

STR loci are important genetic markers for human identity testing. Typically STR genotypes are determined by amplifying target loci with PCR, determining the lengths of the PCR products by referencing their mobility to a DNA standard in capillary gel electrophoresis, and then making allele assignments by comparison to the electrophoretic mobility/lengths of an allelic ladder. With this approach, sequence variants associated with complex repeat structures or single nucleotide polymorphisms (SNPs) are not recognized as distinct alleles. Mass spectrometry provides an alternative analysis of PCR products where mass and base composition are the basis for the allele assignment. Importantly, electrospray ionization-mass spectrometry (ESI-MS) can measure PCR product masses with sufficient accuracy that sequence variants can be identified. We have developed a highly automated PCR assay utilizing ESI-MS analysis to genotype the core STR loci utilized by the forensic communities of the US, Europe, and the UK, and we describe its preliminary evaluation here.

2. Methods

The STR assay was configured in a 96 well plate format with each sample amplified in 5 multiplexed reactions plus 3 non-multiplexed reactions, covering 21 STR loci plus the amelogenin locus. Reactions were formulated as previously described [1]. For PCR, 5ul of sample was added and amplified in an Eppendorf Mastercycler under the following conditions: 96 °C for 10 min; then 40 cycles of 96 °C for 25 s, 60 °C for 45 s, ramp at 5% to 72 °C and hold for 2 min; then 72 °C for 4 min, 96 °C for 10 min, and hold at 4 °C. Plates then were transferred to the Ibis Biosciences PlexID for automated sample processing and ESI-MS analysis. For concordance, samples also were analyzed with the NGM SElect® and Identifiler® kits (Applied Biosystems, Foster City, CA) according to the manufacturer's specifications.

Human DNA was extracted from commercially obtained peripheral blood (Bio-Med Supplies, Carlsbad, CA) with the Gentra Puregene Blood Kit (Qiagen, Valencia, CA), and then polished with organic extraction and precipitation. DNA from six nonhuman species was used to evaluate the specificity of the assay: male dog and male cat (Zyagen, San Diego, CA); *Escherichia coli* DH5α and *Staphylococcus aureus* USA300 (internally prepared); and *Aspergillus oryzae* and *Candida albicans* (ATCC, Manassas, VA).

3. Results

The specificity of the assay was evaluated using DNA from vertebrate, bacterial, and fungal species. None of the nonhuman DNA were reactive when 10 ng per reaction was analyzed; nor did this amount of nonhuman DNA interfere with the analysis of human DNA present as the minor component of a 10:1 mixture. Sensitivity was evaluated with five human DNA samples each analyzed in triplicate at 62.5–500 pg per well. The sensitivity of the

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Table 1
Alleles observed in an analysis of 58 samples with the 22-locus assay.

	Number of unique observations			Total number observed		
	Alleles	SNP alleles	Genotypes	Alleles	SNP alleles	SNP-based heterozygotes[a]
AMEL	2	–	2	97	–	–
CSF1PO	7	–	13	107	–	–
D1S1656	17	10	42	110	67	1
D2S441	11	6	23	104	56	3
D2S1338	24	14	44	112	55	2
D3S1358	16	10	35	107	71	4
D5S818	15	12	31	110	88	9
D7S820	12	5	25	104	11	–
D8S1179	14	7	30	106	61	2
D10S1248	8	–	16	100	–	–
D12S391	31	18	43	114	43	2
D13S317	11	4	32	111	45	7
D16S539	10	3	24	107	15	1
D18S51	12	–	28	111	–	–
D19S433	12	1	26	104	1	–
D21S11	17	8	33	112	23	3
D22S1045	9	–	17	103	–	–
FGA	12	1	29	110	1	–
SE33	29	7	47	109	8	–
THO1	6	–	16	105	–	–
TPOX	6	–	13	95	–	–
vWA	15	8	29	108	26	1

[a] SNP-based heterozygotes had same-length alleles that were heterozygous by base composition.

assay was generally typical of PCR, with full profiles obtained for all replicates at the 500 and 250 pg/well levels; 13 and 9 of the 15 replicates gave full profiles at the 125 and 62.5 pg/well level, respectively. The assay was reproducible, in that the maximum number of allele detections (618) was seen across the replicate samples at both the 500 and 250 pg/well levels. Full concordance of genotypes was seen in comparison to results obtained with the Applied Biosystem kits, excluding the SNP determinations made with the Ibis assay. The allele detections made with this sample set were evaluated to determine the accuracy of the mass measurement, which was calculated as the mass deviation divided by the expected mass; the error distribution and average absolute error were similar to other PCR/ESI-MS assays that have been developed [1]. SNP alleles were seen for most loci in the concordance study, and in some cases were comparatively frequent, as has been observed previously [2,3]. Table 1 lists the alleles observed with 58 samples.

4. Conclusion

An automated assay for genotyping 21 human STR loci plus the amelogenin locus was developed. Initial evaluation of the assay indicates that it performs similarly to other PCR/ESI-MS assays that have been developed, and that its profiles were concordant with existing methods. Notably, sequence variants were observed in most STR loci. SNP alleles were comparatively frequent for some loci, and there was an increase in heterozygosity for a number of them.

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Conflict of interest

The authors who are affiliated with Ibis Biosciences are salaried employees with financial interest in the development and marketing of products and services related to human forensics.

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