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Forensic genetic SNP typing of low-template DNA and highly degraded DNA from crime case samples

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ABSTRACT

Heterozygote imbalances leading to allele drop-outs and disproportionally large stutters leading to allele drop-ins are known stochastic phenomena related to STR typing of low-template DNA (LtDNA). The large stutters and the many drop-ins in typical STR stutter positions are artifacts from the PCR amplification of tandem repeats. These artifacts may be avoided by typing bi-allelic markers instead of STRs. In this work, the SNP*for*ID multiplex assay was used to type LtDNA. A sensitized SNP typing protocol was introduced, that increased signal strengths without increasing noise and without affecting the heterozygote balance. Allele drop-ins were only observed in experiments with 25 pg of DNA and not in experiments with 50 and 100 pg of DNA. The allele drop-in rate in the 25 pg experiments was 0.06% or 100 times lower than what was previously reported for STR typing of LtDNA. A composite model and two different consensus models were used to interpret the SNP data. Correct profiles with 42–49 SNPs were generated from the 50 and 100 pg experiments, whereas a few incorrect genotypes were included in the generated profiles from the 25 pg experiments. With the strict consensus model, between 35 and 48 SNPs were correctly typed in the 25 pg experiments and only one allele drop-out (error rate: 0.07%) was observed in the consensus profiles.

A total of 28 crime case samples were selected for typing with the sensitized SNP*for*ID protocol. The samples were previously typed with old STR kits during the crime case investigation and only partial profiles (0–6 STRs) were obtained. Eleven of the samples could not be quantified with the Quantifiler™ Human DNA Quantification kit because of partial or complete inhibition of the PCR. For eight of these samples, SNP typing was only possible when the buffer and DNA polymerase used in the original protocol was replaced with the AmpFℓSTR® SEfiler Plus™ Master Mix, which was developed specifically for challenging forensic samples. All the crime case samples were successfully typed with the SNP*for*ID multiplex assay and the match probabilities ranged from 1.1×10^{-15} to 7.9×10^{-23}. In comparison, four of the samples could not be typed with the AmpFℓSTR® SEfiler Plus™ kit and the match probabilities were higher than 10^{-7} for another six samples.

1. Introduction

Increasing the typing success of challenging samples is an essential part of all forensic genetic research. The sensitivity of typing assays and the ability to type highly degraded DNA samples is constantly being improved, and today, low-template DNA (LtDNA) analyses [1,2] and assays specifically designed to type highly degraded DNA [3–9] are used for forensic genetic case work by many laboratories.

LtDNA typing is complicated by the occurrence of stochastic phenomena that results in skewed amplification of alleles and loci. The result is frequent heterozygote imbalances and, in the extreme situation, allelic or locus drop-out. The number of drop-outs may be reduced by increasing the sensitivity of the typing assays. Different methods have been used for LtDNA typing of STRs: (1) increased number of PCR cycles (usually from 28 to 34 PCR cycles), or (2) increased number of analyzed PCR products by adding more PCR products, increasing the injection time and/or the injection voltage of the capillary electrophoresis instrument, or (3) post-PCR purification of the PCR products [10–18]. However, the sensitizing methods had two major drawbacks: (1) the signal from stutters increased and (2) the number of drop-in alleles (any identified allele that is not present in the original sample DNA) increased dramatically from approximately zero with the standard protocol to 1–3% of the approved alleles with the sensitized protocols. The majority of drop-in alleles were

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identified in the typical stutter positions. This indicated that the drop-ins were generated by the PCR and were not a result of contamination. By typing the samples two or more times and interpreting the combined results by various consensus models, the number of drop-outs and drop-ins in the generated sample profile were reduced, but not eliminated [10,12,15,16,18]. As a consequence, a "statistical" approach was developed to compensate for the stochastic phenomena of LtDNA analyses [2]. Drop-out and drop-in probabilities were introduced in the calculation of the statistical weight of the evidence, which linked conventional DNA and LtDNA typing in an elegant way and eliminated the need for an "LtDNA threshold", which was very difficult to define. The challenges of the statistical approach were to estimate the drop-out and drop-in probabilities of the sample, locus, or allele under investigation, and although different methods have been tested [19–22], it remains uncertain how these probabilities should be estimated for real case work samples.

The increased sizes of stutters and the increased number of drop-ins observed with the sensitized protocols are phenomena related specifically to the PCR amplification of tandem repeats. Typing of other loci such as single nucleotide polymorphisms (SNPs) and insertion/deletions (indels) are not likely to generate high numbers of PCR artifacts, which should make SNPs and indels more suitable for sensitized LtDNA protocols.

The SNP*for*ID multiplex assay [4] was validated for relationship testing in our ISO17025 accredited laboratory in 2007 [23]. The assay proved to be a valuable supplement to STR typing in cases, where the conclusions based on STRs were ambiguous [24–32]. All 49 SNP loci were amplified in one PCR reaction and the SNPs were detected by two single base extension (SBE) reactions and capillary electrophoresis [4,23,32]. The amplicon lengths ranged from 59 to 115 bps and 38 of the amplicons were shorter than 100 bps. Locus specific guidelines for data analysis based on the peak height(s) of the detected allele(s) were developed during the validation of the SNP*for*ID assay [23]. The guidelines ensured a clear and quantitative distinction between heterozygous and homozygous allele calls and made it easy to identify unusual genotype calls which allowed the analyst to focus on these individual results. Furthermore, they ensured consistency in the data analysis and simplified training of new analysts. Finally, the guidelines made it possible to identify mixtures or contaminated samples [29].

Here, a sensitive SNP typing protocol that increases SBE signal strengths without increasing noise and without affecting the heterozygote balance is introduced. The protocol allows SNP typing of LtDNA with high accuracy and improves the DNA typing success of challenging crime case samples.

2. Materials and methods

2.1. Samples and DNA preparations

For the LtDNA experiments, whole blood samples from 10 Danish individuals and six commonly used reference DNA samples were selected. DNA was extracted from 200 μl whole blood using the the QIAamp DNA blood mini kit (Qiagen). The reference DNA samples were 9947a, 9948a and AmpFℓSTR male control DNA 007 from the AmpFℓSTR PCR amplification kits (LT-AB), the K562 leukaemia cell line (Promega), the male control DNA XY1 (Biotype) and the female control DNA XX74 (Biotype) [33].

A total of 28 crime case samples were selected based on previous STR typing results (Supplementary Table S1). For twelve of these samples, DNA was extracted from two different areas of the sample material and both preparations were investigated. DNA was extracted from the crime case samples either by a standard phenol/chloroform extraction protocol [34] or by chelex-100 resin [35].

2.2. Quantification

All DNA concentrations were determined by real-time PCR using the Quantifiler™ Human DNA Quantification kit (LT-AB) on an AB 7900 (LT-AB) according to the manufacturer's recommendations.

For the LtDNA experiments, quantification was performed in duplicate on the original DNA preparation. The DNA was subsequently diluted to a concentration of 100 pg/μl and quantified again in duplicate. The average concentration of the diluted DNA was 106 pg/μl (range: 63–144 pg/μl).

For the crime case samples, the final dilution of the DNA preparation that could be typed with the SNP*for*ID multiplex was quantified in duplicate (Supplementary Table S1).

2.3. STR typing

STR typing of the crime case samples was performed using the AmpFℓSTR® SGM Plus, AmpFℓSTR® Identifiler or the AmpFℓSTR® SEfiler Plus kits (LT-AB). PCR was performed according to the manufacturer's recommendations using 28, 28 and 30 cycles in the PCR, respectively. PCR reactions were performed in a GeneAmp® PCR system 9700 thermal cycler (LT-AB). A total of 1.5 μl PCR product was mixed with 15 μl Hi-Di™ formamide (LT-AB) and 0.1 μl GeneScan™ 400 HD ROX™ size standard (LT-AB) or 0.3 μl GeneScan™ 500 HD Liz® size standard (LT-AB). Analyses of the amplified PCR products were performed with an ABI 3130*xl* Genetic Analyzer (LT-AB) with 36 cm capillary arrays, POP-4 polymer, and 6 or 20 s injections at 3000 V (LT-AB). Data were analyzed independently by two analysts using GeneScan® analysis software v. 3.7 and Genotyper® analysis software v. 3.7 (LT-AB), and the results were compared. The minimum peak height was set to 50 relative fluorescence units (RFUs) for all dyes. The AmpFℓSTR® SEfiler Plus kit was used for all crime case investigations in our laboratory up to November 2011 [36].

2.4. SNP typing

SNP typing was performed as previously described [23,32] except for two changes in the protocol. For LtDNA typing, the SBE reactions were performed with 100 cycles instead of 30 cycles. For typing of some of the crime case samples (Supplementary Table S1), PCR was performed in 25 μl reactions containing 1–4 μl DNA extract, 10 μl AmpFℓSTR® SEfiler Plus™ Master Mix (LT-AB), 8 mM $MgCl_2$, 700 μM of each dNTP and 0.01–0.17 μM of each primer (DNA Technology). If the signal strength from the crime case samples was low, SBE reactions were performed with 100 cycles. PCR and SBE reactions were performed in a GeneAmp® PCR system 9700 thermal cycler (LT-AB). Two μl SBE products were mixed with 20 μl Hi-Di formamide (LT-AB) and 0.1 μL GeneScan™ 120 Liz® size standard (LT-AB). The SBE products were analyzed by capillary electrophoresis using 3130*xl* Genetic Analyzers (LT-AB) with 36 cm capillary arrays and POP-4 polymer (LT-AB) as previously described [23,32]. Every genotype call was evaluated based on the peak height(s) of the allele(s) according to pre-defined guidelines for allele calling [23]. Genotype calls that did not fulfill the guidelines for allele calling were scrutinized and evaluated individually. If the peak height was less than 300 RFU, the result was only accepted if the noise in that part of the electropherogram was less than 50 RFU. Heterozygous allele calls were never accepted if the peak height ratio was more than two times higher than the maximum or less than half of the minimum value of the pre-defined interval for heterozygous allele calls [23].