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Case report

Application of DIP-STRs to sexual/physical assault investigations: Eight case reports

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ABSTRACT

DIP-STRs are compound markers formed by a deletion/insertion polymorphism linked to a microsatellite. They enable the deconvolution of unbalanced DNA mixtures from two individuals, up to 1000 fold excess of one contributor. In practice, this novel tool allows to test for the presence of a DNA of interest in traces appearing not useful because of the masking effect of the major DNA contributor. Thus far two sets of DIP-STRs have been published: the first set was described as proof-of-principle, while the second set was specifically developed for forensic applications. Here, we report on the first use of these markers in casework to show advantages and limitations in real examples.

Traces, suggestive of containing unbalanced DNA mixtures (beyond standard STR mixture resolution), were selected from eight cases submitted to the Forensic Genetics Unit of the University Center of Legal Medicine of Lausanne-Geneva. Using 18 validated DIP-STRs, two to ten markers were selected for each case. A minor DNA contributor – undetected using conventional STRs – was detected for the trace samples of six cases. DIP-STR results contributed to each case, either by complementing Y-STRs results or by producing novel investigative leads. This was especially true with same sex unbalanced DNA mixtures, female minor/male major unbalanced DNA mixtures or when the source of the DNA mixture was said to come either from the suspect and the female complainant or from his brother and the female complainant. Interestingly, these markers were found to be more sensitive and specific than previously known. Positive results were obtained at 16,000-fold excess of major DNA using few picograms of input DNA, as well as from traces collected several months after the alleged offence. Likelihood ratios assigned to measure the strength of DIP-STRs' DNA evidence were modest (10), when accounted by only two DIP-STRs, and high (10^6) when determined by six markers. In some cases the detection of extra alleles from additional minor DNA contributors or because of extremely unbalanced DNA ratios, limited the interpretation of the results. In conclusion, the DIP-STRs often provide additional value to the analysis of traces that cannot be exploited by the use of standard methods.

1. Introduction

Using autosomal STR markers trace samples recovered from crime scenes can produce challenging DNA mixtures with several individuals contributing different amounts. These may include the minor DNA from the alleged offender and the major DNA from the complainant or the other way around. Indeed, autosomal STRs represent the mainstay forensic markers for human identification purposes due to their high discrimination power and well-established national DNA databases [1]. However, when analyzed with conventional Polymerase Chain Reaction (PCR) and capillary electrophoresis, these markers are limited in resolving unbalanced two-source DNA mixtures where the minor contributor can only be detected when the major DNA does not exceed a 10-, 20-fold excess [2,3].

In addition to autosomal STR's highly sensitive Y-chromosome STRs can be used for investigating mixed biological samples (*e.g.* in sexual assaults) in which the DNA of a male contributor is mixed to high levels of female DNA. Though a specific sex mismatch is required for the application of Y-STRs [4,5], they can be used to discriminate unrelated male individuals [6] and to reconstruct paternal relationships between males [7]. A significant improvement in discriminating paternally related individuals was reported by the 13-rapidly mutating Y-STR assay, which enables the

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differentiation of up to 50% father-son pairs and 60% of brother pairs [8,9].

To further contribute to the resolution of unbalanced DNA mixtures, we developed a highly specific and sensitive genetic marker, denoted DIP-STR [10]. The innovative feature of the DIP-STR is represented by the combined analysis of a DIP (Deletion/Insertion Polymorphism) and a closely linked STR polymorphism. PCR primers overlapping the deleted/inserted sequence (S or L) produce allele-specific amplifications of one or two minor DIP-STR haplotypes comprising the DIP allele type that is not shared with the major DNA. Such selective PCR amplifications reach a high level of specificity so that a minor DNA can be detected in the presence of up to 1000-fold excess of major DNA. Finally, that the DIP-STR loci characterized autosomal, constraints of sex mismatch between the two contributors are eliminated and the product rule can be applied in order to assess the value of the results.

To promote the use of DIP-STRs in casework an additional forensic set of 10 markers was recently developed and the performance of this new type of marker has been evaluated on contact traces simulated using substrates representative of crime-related objects [11,12]. Finally, a Bayesian framework is available for the evaluation this type of evidence [13,14].

The aim of this work is to report on the first application of DIP-STR markers on a series of casework DNA samples taken from complainant or defendants in sexual/physical assault submitted to the Forensic Genetics Unit of the University Center of Legal Medicine of Lausanne-Geneva. Eight casework studies described hereafter show that DIP-STRs can be an effective tool in forensic investigations and also highlight some of the current limitations to their use.

2. Material and methods

2.1. Selection of forensic samples from casework

Traces were selected from the casework submitted in our laboratory. Notably, these included traces collected on complainants (body or clothes) or defendant of sexual/physical assaults committed across different French-speaking Swiss cantons. Official permission to further analyse crime related DNA samples and to present the corresponding data was obtained by the Police and the General Prosecutor authorities.

An overview of the circumstantial information relative to the specific casework traces is presented in Section 3, in full compliance with the maintenance of privacy of the individuals involved. Overall, these examples represent cases where the DIP-STR genotyping was proposed to exploit further a biological trace remained underused after conventional forensic methods or alternatively to complement the results obtained with classical methods.

2.2. Presumptive tests

These included the immuno-chromatographic membrane assay SERATEC PSA Semiquant test (SERATEC® GmbH Goettingen, Germany) for seminal fluid detection and the Christmas Tree stain for spermatozoa cytological detection [15].

2.3. DNA extraction and quantification

REFERENCES buccal and blood samples of complainants and defendants were collected by Hospital and Police Cantonal authorities. Buccal samples were extracted using the SwabSolution™ Kit (Promega, AG, Dübendorf, Switzerland) or Chelex®-100 resin (Bio-Rad, AG, Cressier, Switzerland) by the addition of 300 μL SwabSolution™ Reagent, and 350 μL 5% Chelex solution and 2 μL of 17.1 mg/mL Proteinase K to the sample tubes containing the trace, respectively [16]. Reference blood samples were extracted using the QIAamp DNA Mini Kit (Qiagen AG, Basel, Switzerland) according to the manufacturer's protocol and eluted in 100 μL final volume.

Trace samples were extracted either manually or using automated DNA extraction platforms and eluted in 50 μL final volume. Depending on when the analysis took place, different techniques were used. In particular, trace from case 1 was extracted on the QIAsymphony Automated DNA Extraction Platform (Qiagen). QIAshredder spin columns (Qiagen) were used for lysate homogenization and the DNA Investigator® QIAsymphony Kit (Qiagen) for DNA purification according to the manufacturer's protocol accompanying the instrument. Traces from cases 2, 3, 4, 5 and 6 were extracted using the QIAshredder/QIAamp DNA mini protocol (Qiagen) [17] and then concentrated using the Amicon Ultra-0.5 30 K device (Merck Millipore, Shaffhausen Switzerland) following the manufacturer's protocol.

The automated lysis and DNA extraction of traces from cases 7 and 8 was performed on the high throughput Microlab AutoLys STAR/Microlab® IDSTARlet workstation systems (Hamilton, AG, Bonaduz, Switzerland) using the PrepFiler™ Automated Forensic DNA Extraction Kit (Applied Biosystems) according to the manufacturer's protocol.

Lastly, DNA samples from case 1 and 3 were quantified with the Quantifiler Duo Human DNA Quantification Kit (Applied Biosystems) while samples from case 2 and 4 to 8 with the Investigator Quantiplex HYres™ assay (Qiagen) on an AB 7500 Real-time PCR system (Applied Biosystems) and analyzed using the HID Real-Time PCR Analysis Software v1.2 (Applied Biosystems).

2.4. PCR conditions

Autosomal STRs and Y-STRs profiles of both complainants and defendants were produced using the commercial NGM SElect™ (Applied Biosystems), PowerPlex®ESI 17 System (Promega AG, Dübendorf, Switzerland) and Powerplex®Y23 (Promega) PCR assays. Reference DNA and trace samples were amplified at 27 and 30 PCR cycles, respectively using autosomal STR markers and at 25 and 30 PCR cycles using the Y-STR PCR kits on a GeneAmp 9700 (Applied Biosystems) thermal cycler. For traces, autosomal STRs were amplified in a 25 μL PCR volume using input DNA of 10, 5, 4, 3, 2 μL at concentration range of 0–0.063, 0.063–0.125, 0.125–0.250, 0.250–0.500, 0.500–1.000 ng/μL, respectively. DNA samples of higher concentration were diluted down to 1 ng/μL. Y-STRs were amplified in 25 μL PCR volume using at most 10 μL input DNA for 0.5 ng/μL DNA concentration.

For DIP-STR analyses, informative markers were first selected for each case by using two DIP multiplex reactions covering the first [10] and second [11] marker sets. These tests allow the selection of markers in which the major and minor DNA contributors are opposite DIP homozygous (LL/SS or SS/LL) or markers in which the major contributor is homozygous (LL or SS) and the minor is DIP-heterozygous. DIP-STR haplotypes analyzed by using PCR primers overlapping the DIP sequence on one side (either L-DIP or S-DIP primer) and downstream the STR (STR primer) on the other side enables the characterization of two minor DNA haplotypes in the first case and one minor DNA haplotype in the second case. Both DIP multiplex sets were amplified in 20 μL PCR final volume. This included 1 × PCR Buffer containing 1.5 mM $MgCl_2$ (Applied Biosystems), 250 μM dNTP (Applied Biosystems), 1.2 U AmpliTaq Gold DNA Polymerase (Applied Biosystems), 0.2 μM each primer (Applied Biosystems) and 0.5 ng DNA. PCR thermal cycling conditions of DIP markers of the first set [10] were as follows: 5 min at 95 °C, 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C for 30 PCR cycles and a final extension of 30 min at 72 °C. The same conditions were applied to the