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Short Report

STR typing of archival Bouin's fluid-fixed paraffin-embedded tissue using new sensitive redesigned primers for three STR loci (CSF1P0, D8S1179 and D13S317)

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Abstract

Three new mini-STR primer sets are suggested for three conventional STRs, CSF1P0, D8S1179 and D13S317, included in multiplex PCR kits commercially available and commonly used for DNA typing in forensic applications. The primer pairs for the three loci were redesigned in order to reduce or eliminate the flanking regions of the polymorphism obtaining amplification products, which have dimensions less than 120 bp in size. A comparison of results for typing carried out with the newly designed primers on DNA extracted from 100 blood samples provided by healthy donors, previously typed with conventional STRs, showed no genotype difference underlining their precision and reproducibility. The forensic usefulness of the new mini-STR primers was evaluated on highly degraded DNA from casework samples (e.g. archival post-mortem Bouin's fluid-fixed paraffin-embedded tissue specimens) for which commercial STR kit had proven inefficient.

Keywords: STRs; CSF1P0; D8S1179; D13S317; Degraded DNA; Bouin's fluid-fixed paraffin-embedded tissue

1. Introduction

Paternity testing is generally ascertained using blood samples or other biological material obtained from the subjects involved in the analysis. Archival post-mortem or biopsy paraffin-embedded tissues are potential samples for DNA extraction for subsequent genetic testing for forensic applications. However, the DNA extracted from these samples is often disposable in low amount (depending on the nature and quantity of the tissue included) and degraded to various extents due to the fixing and inclusion conditions (type and time of fixing, time and storage conditions). Therefore, the DNA extracted from these samples may be characterized by low copy number where brief sequences of the degraded target DNA predominate, generally smaller than the STRs conventionally used in the PCR.[1] This negatively impacts on the amplification process leading to ambiguous results (artificial formations such as allele drop-out) or failure of the PCR process.

Previous studies showed that DNA artificially degraded leads to relatively stable fragment lengths up to 200 bp, that when submitted to PCR amplification using STR multiplex kits, only smaller STR are amplified, resulting in partial STR profiles.[2,3]

The Authors of this study have noted that from archival post-mortem Bouin's fluid-fixed paraffin-embedded tissue specimens stored at room temperature for more than 10 years, it is obtained highly degraded DNA with no significant number of intact DNA fragments greater than 120 bp. This it was inferable from the failure of the processes of amplification with AmpFlSTR Identifiler® PCR Amplification (Applied Biosystems, Foster City, CA) that did not provide any genetic profile, also when the number of

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PCR cycles was increased from 28 to 34, with the only except for amelogenin marker, which is known to have dimensions of 106–112 bp.

An approach to trying to recover information from degraded DNA samples is to reduce amplicon lengths of PCR products by moving primers in as close as possible to the STR repeat region.[4–9]

The need to obtain a genetic profile, although limited to a few STRs by the low amount of highly degraded DNA extracted from included tissue, has led the authors to convert three conventional STRs into Mini-STRs by re-engineering, so that the primers are built close to the repeat region as recommended by EDNAP and ENFSI groups.[10]

In this case the attention was focused on STR loci, characterized by a relatively low number of repetitive units (<20), so it is possible to obtain fragment lengths less than 120 bp. For that reason, we decided to use CSF1P0, D8S1179 and D13S317 that are present in many commercial STR multiplex amplification kits (e.g. AmpFlSTR Identifiler® PCR Amplification, AB) and that are included in the standard STR loci of nationwide DNA intelligence databases in various countries.[11]

2. Materials and methods

DNA was extracted from 100 fresh blood samples provided by healthy donors (previously typed with AmpFlSTR Idenfiler® PCR Amplification) using GenomicPrep Blood DNA Isolation Kit (Ge Healthcare Bio-Sciences, Uppsala, Sweden).

DNA was extracted from five archival Bouin's fluid-fixed paraffin-embedded tissue specimens (stored at room temperature, since 1990) following manual microdissection of 10 μm thick unstained sections. The microdissection tissue fragments were de-waxed through further xylene and ethanol washes. After air drying, the tissue pellets were digested using DNA IQ™ system (Promega, Madison, WI, USA) following the manufacturer's instructions. DNA samples were measured spectrophotometrically to determine quantity and quality. Cell line K562 (Promega) was serially diluted from a concentration of 1 ng to 30 pg for sensitivity and peak imbalance study.

The three new mini-STRs were amplified in singleplex and their primer sequences are given in Table 1. The amplification conditions were the same for the three STRs.

Each PCR reaction was carried out in a total volume of 25 μL containing 10 μL DNA, 1× PCR Buffer II, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.4 μM of each primers and 1.5 units of AmpliTaq DNA polymerase (5 U/μL) (AB, Foster City, CA, USA). The primers were synthesised by Tib Molbiol (Genoa, Italy), and the forward primer from each primer set was labelled at the 5′-end with the fluorescent dye Cy5. The three STR loci were amplified at the same conditions: 95 °C for 2 min, followed by 34 cycles at 95 °C for 45 s, 54 °C for 45 s, 72 °C for 30 s, and a final extension step at 72 °C for 10 min. All reactions, together with positive and negative control samples, were performed in a GeneAmp® PCR System 9700 Thermal Cycler (AB, Foster City, CA, USA).

Samples were set up for electrophoresis by combining 5 μL of each PCR product to 3 μL of loading dye and 2 μL 50–500 bp Size Standard (Amersham Biosciences). The same fluorescently labeled size standard (50–500 bp) was also used as an external standard to compensate for any mobility shift between the lanes. After denaturation at 95 °C for 3 min and subsequently chilled on an ice block for 3 min, the samples were resolved through electrophoresis in a 6% w/v polyacrylamide gel with a 19:1 ratio of acrylamide/bisacrylamide (Ready Mix Gel ALF grade, Amersham Biosciences, Buckinghamshire, England). Electrophoresis was carried out by Automatic Laser Fluorescent (ALFexpress) DNA sequencer (Pharmacia-Biotech, Uppsala, Sweden) at 1450 V, 38 mA, 45 W and 48 °C with laser power at 3 mW for 100 min. Raw data were assigned fragment sizes in base pairs with reference to the internal standard, using analysis Fragment Manager Software V 1.2 run under OS/2. Genotype assignment was done by comparison with sequenced allelic ladders and allele designation following the recommendations of the DNA Commission of the ISFH.[12]

Furthermore, DNA extract from five archival Bouin's fluid-fixed paraffin-embedded tissue was amplified with AmpFlSTR Identifiler® PCR Amplification in accordance with the instructions provided by the manufacturer (Applied Biosystems) and the numbers of PCR cycles were

Table 1
Primer sequences and product sizes used in this study

Locus	Mini-STR primers (5′ → 3′)	Allele range[a]	Mini-STR size (bp)	STR kit product size (bp)	Size reduction (bp)
CSF1P0	5′-CATAGATAGAAGATAGATAG-3′ 5′-CCTGTTCTAAGTACTTCC-3′	6–16	66–106	306–346 (Identifiler)	240
D8S1179	5′-GTATTTCATGTGTACATTCG-3′ 5′-GATTATTTTCACTGTGGGG-3′	7–19	71–119	124–172 (Identifiler)	53
D13S317	5′-CTATCTGTATTTACAAATAC-3′ 5′-CAGAAAGATAGATAGATG-3′	5–16	64–108	205–249 (Identifiler)	141

[a] As reported in the literature; see STR Fact Sheets in STRBase (http://ibm4.carb.nist.gov:880/dna/home.htm).