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# STRs, mini STRs and SNPs – A comparative study for typing degraded DNA

Tim Senge [a,b,*], Burkhard Madea [a], Anke Junge [a,1], Markus A. Rothschild [b], Peter M. Schneider [b]

[a] Institute of Legal Medicine, University of Bonn, Stiftsplatz 12, 53111 Bonn, Germany
[b] Institute of Legal Medicine, University Hospital, University of Cologne, Melatengürtel 60-62, 50823 Cologne, Germany

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ABSTRACT

Short tandem repeat (STR) systems are the most powerful and widely used genetic marker systems in forensic DNA typing. Optimized amplification conditions and PCR reagents in combination with laser fluorescence based detection methods have increased the sensitivity and decreased the detection threshold down to approximately 100 pg. The quality of human DNA from forensic samples can be influenced by environmental factors. These may cause different degrees of degradation which have a negative impact on the amplification process especially of STR systems with large amplicons. Therefore, methods which need only small amplicon sizes to detect DNA markers are a better choice for typing degraded DNA. Here we report investigations on different types of DNA markers and typing methods which should all be applicable for analysing degraded DNA. These are two commercially available mini STR kits and five SNP markers which were analysed with two self established assays, a 5′ nuclease assay and a minisequencing (SNaPshot) assay. The investigations comprised sensitivity studies, different types of stain material, as well as intact and degraded DNA. Results indicate that mini STRs are superior to standard STR typing methods, especially for typing old stain material with small amounts of degraded DNA. SNP typing based on the minisequencing (SNaPshot) assay achieved a better success rate in typing aged blood and saliva stains compared to standard STRs and SNP typing using the 5′ nuclease assay.



## 1. Introduction

STR (short tandem repeat) systems are highly useful markers for forensic stain typing and paternity testing. Following extensive validation, multiplex STR typing kits have been developed for the simultaneous amplification of up to 16 STR loci. The current commercially available multiplex kits generate amplicon sizes ranging from 100 up to 450 bp. The optimization of PCR reaction conditions in combination with laser fluorescence based detection systems has reduced the detection threshold to less than 100 pg genomic DNA, thus minimal DNA traces can be typed successfully [1–4]. However, STR typing is limited by the degree of DNA degradation caused by environmental factors, and depends on the length of the PCR amplicons. DNA degradation may cause artefacts such as preferential amplification, allele drop out and/or locus drop out [5–7]. The efficiency of typing degraded DNA can be increased by selecting primers which are located more closely to the repeat regions thus reducing the amplicon sizes. In the past numerous so called mini STRs were developed and established for routine case work [8–12]. More recently, single nucleotide polymorphisms (SNPs) were analysed for their applicability in forensic DNA typing [13–15]. Since SNPs are characterized as variations of a single base at a specific position in the genome, these systems are preferable to achieve the ultimate lower limit of small amplicons (about 40–50 bp) [7]. Today, numerous SNP genotyping methods exist including the 5′ nuclease assay and minisequencing [13] which are used in this study. Due to these features, SNPs have become valuable markers for forensic DNA typing especially in cases of degraded DNA [7,14,15].

The aim of this study was to investigate which marker system and typing method is the best choice for typing degraded DNA. Sensitivity and degradation studies were performed using both the mini STR kits MPX-SP1 and MPX-SP2 (SERAC GmbH) as well as five SNP markers which were analysed with self established assays applying the 5′ nuclease assay and minisequencing (SNaPshot™). The results were compared with those of the standard multiplex STR kit Mentype Nonaplex QS (Biotype AG).

## 2. Materials and methods

### 2.1. Human genotyping control samples

Genomic DNA was extracted from blood or buccal swab samples obtained with informed consent from randomly selected

* Corresponding author. Present address: Labor für Abstammungsgenetik – Dres. med. M. Krause & K.-G. Heide, Steenbeker Weg 33, 24106 Kiel, Germany. Tel.: +49 (0) 431 22010600; fax: +49 (0) 431 22010699.
*E-mail address:* timsenge@googlemail.com (T. Senge).
[1] Present address: IMoGen GmbH, Heilsbachstraße 17, 53123 Bonn, Germany.

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individuals of Caucasian origin tested previously in routine paternity cases.

### 2.2. DNA extraction

DNA extraction for all samples – with the exception of the environmental studies – was performed using 97.5 µl 5% Chelex 100 solution (Chelex 100 Resin, Bio-Rad Laboratories GmbH) [16] and 2.5 µl proteinase K (20 mg/ml, Roche GmbH).

DNA extraction for samples of the environmental studies was done in intervals of 14 days using the "All tissue" DNA kit (GENIAL GmbH) according to the manufacturer's instructions.

### 2.3. DNA quantification

Extracted DNA was quantified using the Quantifiler Human DNA Quantification kit (Applied Biosystems) according to the manufacturer's instructions. The analysis was performed on the ABI Prism 7000 Sequence Detection System (Applied Biosystems) and the ABI Prism 7000 SDS software v. 1.0.1.

### 2.4. PCR amplification

PCR amplification was carried out using the multiplex STR kits MPX-SP1, MPX-SP2 (SERAC GmbH) and Mentype Nonaplex QS (Biotype AG) according to the manufacturer's instructions. The optimal amount of template DNA for amplification was 0.5 ng (MPX-SP1 + 2 and Mentype Nonaplex QS). DNA was amplified using a TRIO Thermoblock (Biometra GmbH).

### 2.5. Electrophoresis

Electrophoresis was carried out using an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with a 47 cm capillary and POP6 polymer. From each sample, 1–5 µl were mixed with 12 µl HiDi formamide (Applied Biosystems) and 0.5 µl of the internal size standard genRES LS 350 ORN (MPX-SP1 + 2) or DNA Size standard 550 ROX (Mentype Nonaplex QS). After denaturation (5 min, 95 °C), the samples were cooled on ice and immediately transferred to the autosampler. The data were collected using the ABI Prism 310 collection software (v. 3.0.0) and analysed with the 310 Genescan (v. 3.7.1) and Genotyper (v. 3.7 NT) software.

The minimal peak height threshold for analysis was 50 RFU. Another critical factor was that it should always be possible to assign each peak to the correct allele. If the height of a certain peak was above 50 RFU, but the peak could not be distinguished clearly from the background, this peak was not analysed to prevent incorrect allele calling.

### 2.6. Single nucleotide polymorphisms (SNPs)

The 5′ nuclease method was carried out using TaqMan Universal PCR Master Mix with UNG (Applied Biosystems) and by carrying out five self established 5′ nuclease assays (see Tables 1 and 2) with an ABI Prism 7000 Sequence Detection System (Applied Biosystems) according to the manufacturer's instructions. Real time PCR amplification was carried out at 95 °C – 10 min, 1 cycle; 95 °C – 15 s, 60 °C – 1 min, 40 cycles. Before and after the PCR, a single cycle of 60 °C – 1 min was carried out to read the respective fluorescence values. The increase of fluorescence was determined by calculating the difference of these two values. Data were analysed using the ABI Prism 7000 SDS Software v. 1.0.1 (Applied Biosystems).

PCR primers, MGB probes and minisequencing (SNaPshot) primers were designed using the ABI Prism Primer Express Software v. 2.0.0 (Applied Biosystems) (Tables 1–3).

**Table 1**
PCR primer sequences for 5′ nuclease and minisequencing (SNaPshot) assays.

| SNP | PCR primer sequences (5′–3′) | Primer concentrations for 5′ nuclease assays (Minisequencing PCR) |
|---|---|---|
| TSC0741184 | F: CAATCCACCTCCAGTTGTCCTT<br>R: TGGCATGTTTTCAGAGCATCA | 0.9 µM (0.4 µM) |
| TSC0582423 | F: CCTCCCAAGCCCATTTTAATTT<br>R: AACACAAGCCTTCATTTTCCAAA | 0.9 µM (0.4 µM) |
| TSC0171847 | F: TGGATGATTTCACATGTCACATTC<br>R: CCCGTCCGTCTCCATCCT | 0.9 µM (0.6 µM) |
| TSC0126548 | F: TTGGCTTTCCCCTTATTTCTCTAG<br>R: CCAGTGGATGCGCCAGTT | 0.9 µM (0.4 µM) |
| TSC0191459 | F: TGGGAAATATAAGGCAGGTATCCT<br>R: CAGGCTAACATGGAAGAAGAGCTT | 0.9 µM (0.4 µM) |

F = forward primer; R = reverse primer.

**Table 2**
Allele-specific hybridization probes for the 5′ nuclease assays.

| SNP | Sequences of allele-specific probes (5′–3′) | Label | Concentration (µM) |
|---|---|---|---|
| TSC0741184 | TCTTCTC<u>A</u>CTAAGAACTAA<br>CTTCTC<u>G</u>CTAAGAAC | FAM<br>VIC | 0.2 |
| TSC0582423 | ATAGCTGTC<u>T</u>AACTATTG<br>TAGCTGTC<u>C</u>AACTATT | FAM<br>VIC | 0.2 |
| TSC0171847 | TCCAGG<u>T</u>CACCAGTC<br>TTCCAGG<u>C</u>CACCAGT | FAM<br>VIC | 0.2 |
| TSC0126548 | ACTACTTTG<u>C</u>CTTCAAG<br>ACTACTTTG<u>G</u>CTTCAAG | VIC<br>FAM | 0.2 |
| TSC0191459 | CACATCAA<u>T</u>AGGAACTG<br>ACATCAA<u>C</u>AGGAACTG | FAM<br>VIC | 0.2 |

The SNP position is underlined.

Minisequencing was carried out using the SNaPshot™ kit (Applied Biosystems) according to the manufacturer's instructions. Standard PCR amplification was carried out at 95 °C – 1 min, 57 °C – 1 min, 72 °C – 1 min, 30 cycles; 72 °C – 5 min, 1 cycle. The reaction mix consisted of 1.5 mM $MgCl_2$ (Serac GmbH), 0.2 mM dNTPs (GE Healthcare), 1× PCR-buffer, 1 U genRES Plus DNA Polymerase (both from Serac GmbH) and 0.4 µM (TSC0741184, TSC0582423, TSC0126548, TSC0191459) or 0.6 µM (TSC0171847) PCR primers (Applied Biosystems) in a total volume of 25 µl. Minisequencing was performed at 96 °C – 10 s, 50 °C – 5 s, 60 °C – 30 s, 25 cycles in a PTC-220 DNA Dyad Peltier Thermal Cycler (MJ Research Inc.). Electrophoresis of minisequencing (SNaPshot) reaction products was carried out also using the ABI Prism 310 Genetic Analyzer with a 47 cm capillary and POP6. For loading, 0.5 µl of each sample were mixed with 8 µl HiDi formamide (Applied Biosystems) and 0.5 µl of the internal size standard GeneScan LIZ 120. After denaturation (5 min, 95 °C), the samples were cooled on ice and immediately transferred to the autosampler. The data were collected using the ABI Prism 310 collection software (v. 3.0.0) and analysed with the 310 Genescan (v. 3.7.1) software.

5′ nuclease and minisequencing (SNaPshot) assays both use identical PCR primer sequences. Therefore, both assays share the same amplicon range for detecting SNPs. A comparison of the amplicon ranges of STRs, mini STRs, and SNPs used in this study is shown in table 4.

### 2.7. Studies on fresh blood and saliva samples

#### 2.7.1. Multiplex kits

The STR genotypes of 50 randomly selected unrelated persons were determined using the above mentioned extraction, amplification and analysis parameters.