Extraction and PCR amplification of DNA from hair shafts

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Introduction

The increasing occurrence of single hair shafts without recognizable roots in forensic cases requires the optimization of extraction and amplification parameters. The aim of this study was therefore the determination of the success rate of amplification dependent on the amount of hair shafts and the STR (short tandem repeat) system applied.

Materials and methods

Blood samples were extracted as described previously (Brinkmann et al. 1991). The studies were carried out on freshly plucked hair from the back of the head from 13 different persons. The first 2 mm at the root were removed and the hair shafts divided into 5 cm pieces each. 1, 5 and 15 standard hair shafts were investigated.

Extraction protocol:
Hair shafts were cleaned with 100% ethanol (30 min, rt) followed by treatment with proteinase K (250 ng/µl) and 0,188 M DTT (50°C, overnight), phenol-chloroform extraction and centricon 100 purification.

Amplification protocol:
1. HumD21S11 (Sharma and Litt 1992)
5 µl centricon eluate, 1.5 mM $MgCl_2$, 2 µl 10xPCR buffer (Promega, USA), 1 µM of each primer, 100 µM each dNTP, 1.5 U Taq polymerase (Promega, USA) diluted with water to a final volume of 25 µl.

94°C-15s, 62°C-30s, 72°C-75s; 31 cycles
(Biometra Triothermoblock, Germany)
Reamplification of 1 µl of the PCR assay was carried out for 10 and 15 cycles as described above.

2. For HumVWA (Kimpton et al. 1992) and HumMBP (Polymeropoulos et al. 1992)
PCR amplification was carried out with 5 µl centricon eluate according to a previous publication (Möller et al. 1992, in press) except that the cycle number was increased from 30 to 32 for HumVWA. Reamplification was carried out with 10 and 15 cycles for HumMBP.
The electrophoretical separation of the amplified fragments was carried out according to Möller et al. (1992, in press). Visualization of the bands was performed by silver staining (Budowle et al. 1991).

3.HumTH01 (Edwards et al. 1992)
-first amplification: 5 µl centricon eluate, 1.5 mM $MgCl_2$, 2 µl 10xPCR buffer (Promega, USA), 0.24 µM of each primer (Edwards et al. 1992, primer 1 fluorescence-labeled with FAM), 100 µM each dNTP, 2.5 U Taq polymerase in a final volume of 25 µl.

95°C-45s, 60°C-30s, 72°C-30s, 30 cycles (Perkin Elmer 9600)

Semi-nested PCR was carried out with 20 cycles, 2 µl of the PCR assay and the primers developed by Gill et al. (1992, primer 1 fluorescence-labeled with FAM) using the PCR conditions as described above. PCR fragments were separated on a 6% denaturing gel and analysed on the ABI 373A Sequencer using the Genscan software 672 and the internal standard Genscan 2500 labeled with ROX.

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Results and discussion

Reamplification was the most effective modification for HumD21S11 and HumMBP.

After 31 cycles no PCR product could be obtained for HumMBP with DNA extracted from 5 hair shafts. After reamplification with 10 and 15 cycles DNA fragments within the allelic range could be demonstrated for locus A and locus B (Fig.1). Increasing the cycle number from 10 to 15 produced ladder bands caused by polymerase slippage and/or heteroduplex formation (Fig. 1).

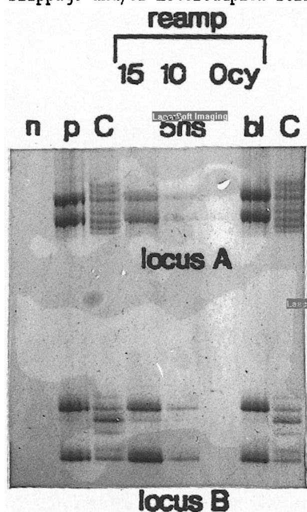

Fig. 1: HumMBP: Amplification patterns of DNA extracted from 5 hair shafts after 31 cycles (0 cycles of reamplification) and after reamplification with 10 and 15 cycles
C = allelic ladder
bl = blood DNA
p = positive control sample
n = negative control sample

An increase from 30 to 32 cycles improved the results for HumVWA. The pattern of 1 and 15 hair shafts matched with the correspondending root pattern whereas 5 hair shafts gave ladder bands (Fig. 2).

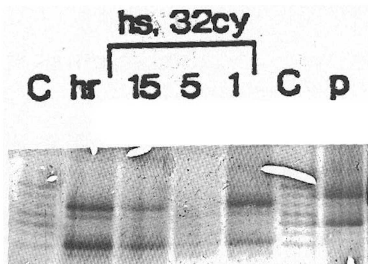

Fig. 2: HumVWA: Amplification patterns of DNA extracted from 1, 5 and 15 hair shafts after 32 cycles
C = allelic ladder
hr = DNA extracted from 1 hair root
p = positive control sample

Using the fluorescence detection system and semi-nested PCR HumTH01 was the most successful STR system and highly sensitive. Clear amplification patterns could be demonstrated with DNA extracted from 1 hair shaft (Fig. 3). The peak heights of blood DNA after standard PCR and 1 hair shaft after semi-nested PCR were similar indicating that the application of semi-nested PCR is a powerful tool to maximize the sensitivity.

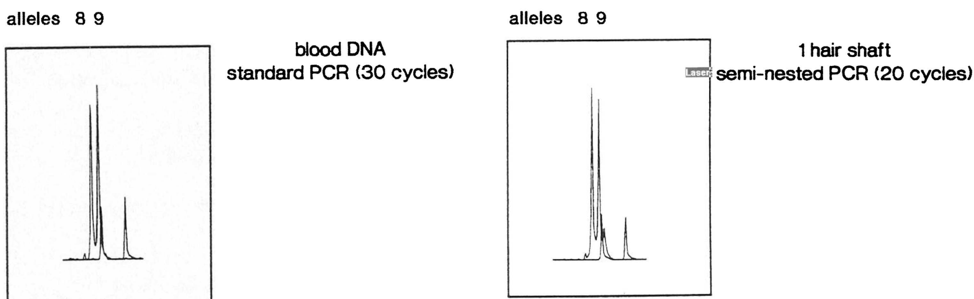

Fig. 3: HumTH01: Amplification patterns of blood DNA after standard PCR (30 cycles) and DNA extracted from 1 hair shaft after semi-nested PCR (20 cycles)

Determination of the success rates showed that, with the exception of HumD21S11, an increase in the amount of hair shafts is in general paralleled with an increase in the success rate (Fig. 4). For HumTH01 it increases from only 15% for 1 hair shaft up to approximately 50% for 15 hair shafts. HumVWA and HumMBP have comparable success rates which increased from about 20% for 5 hair shafts up to approximately 40% for 15 hair shafts.

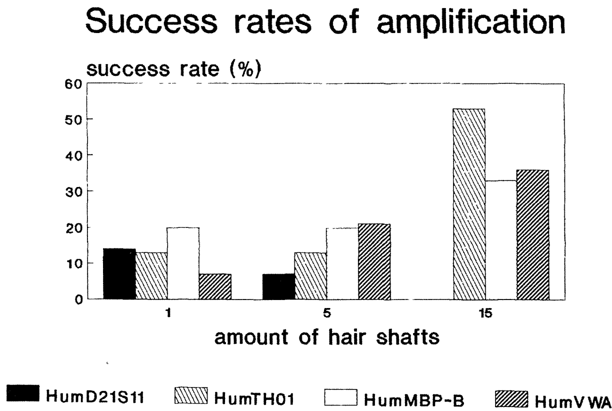

Fig. 4: Success rates of PCR amplification for different amounts of hair shafts using the STR systems HumD21S11, HumTH01, HumMBP-B and HumVWA

Successful DNA typing was dependent on the quantity and quality of hair shafts and the system applied. Due to the low amount of template DNA one has to consider that artefacts such as allelic drop out, additional bands and incorrect amplification patterns could occur. For the future, the aim must be to eliminate these artefacts and to increase the success rate by applying further modifications.

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