Investigations on the Forensic application of 4 AMPFLP systems

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INTRODUCTION

One major problem with stain examinations is that the amount of biological material is often too small and the extracted DNA is highly degraded due to the environmental conditions. It is therefore highly probable that only PCR can be successfully used for DNA analysis and individualisation.
Recently 4 amplifiable fragment length polymorphisms have been under intensive investigation:
MCT118 - APOB - YNZ22 - COL2A1
These systems seem to be very powerful tools for resolving the problem of highly degraded DNA. But before they can be established in casework, basic experience is necessary to prove the reliability of the results.

MATERIALS AND METHODS

The investigated blood samples were obtained from paternity cases from the Münster area. DNA was extracted from EDTA blood as previously described (Brinkmann et al. 1991).
Chelex extraction from blood stains was carried out as described by Walsh et al (1990).
The vaginal swabs were taken 1-2 days after sexual intercourse and extracted using "single lysis" or "mild preferential lysis" (Wiegand et al. 1991 in press).
The extraction of the cigarette butts (crime case 1) was carried out using proteinase K, phenol-chloroform-isoamylalcohol (24:24:1) and ethanol precipitation.
PCR-amplification was performed using published conditions and primers:
MCT118 (Budowle et al. 1991)
APOB (Boerwinkle et al. 1989)
COL2A1 (Wu et al. 1990)
YNZ22 (the following conditions were used;B. Budowle, pers. com.)
Primer sequence:
5'-AAACTGCAGAGAGAAAGGTCGAAGAGTGAAGTG-3'
5'-AAAGGATCCCCCACATCCGCTCCCCAAGTT-3'

temp.:	94 /	63 /	72 °C
time :	1	1	6 min
cycl.:	27		

Thermocycler: Triothermoblock (Biometra, FRG)

Amplification was carried out with 2.5 U Taq polymerase (Promega corporation, USA) in 10mmol Tris-HCl, pH 8.3, 50 mmol KCl, 1.5 mmol $MgCl_2$, 0.1% Triton-X, 0.5 μM each primer and 200 μM each dNTP. The total volume was 50 μl with the addition of 30 μl oil overlay.
The electrophortical separation of the amplified fragments was carried out in polyacrylamide gels (6% T, 3% C; thickness 400 μm) with piperazine diacrylamide as cross-linker (Budowle et al. 1991) using a discontinous buffer system (Allen et al. 1989). The separation distance was 10 cm. Visualisation of the bands was performed by silver staining (Budowle et al. 1991).

RESULTS AND DISCUSSION

The most common stain materials are bloodstains, mixtures of vaginal cells, semen and sometimes hairs. Chelex extraction from blood stains (3 mm^2 area on cotton), which were stored at room temperature over different time periods, clearly showed visible bands for each stain (Fig. 1).

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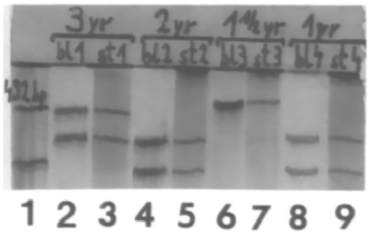

Fig. 1: Comparison between amplified blood DNA and corresponding Chelex extracted bloodstains after different storage time. System: MCT118
lane 1 : 123 bp ladder (Gibco-BRL, UK)
lane 2, 4, 6, 8: phenol-chloroform extracted blood DNA
lane 3, 5, 7, 9: Chelex extracted bloodstains (5-10 ng DNA template)

A very problematical area is the application of PCR to mixtures of cells or body fluids which are present in different proportions and is a problem mainly encountered with secretions from sexual organs. Firstly it is necessary to know the weakest concentration which can be detected.
If different ratios of a mixture of 2 sperm populations were amplified, the recognition of the weakest component was possible down to a minimal concentration of 10 % (Fig. 2). But in these cases disadvantageous effects can also occur: In a 1 : 5 mixture ratio of spermatozoa from 2 individuals, the larger fragment was virtually suppressed (Fig. 3, lane 4).

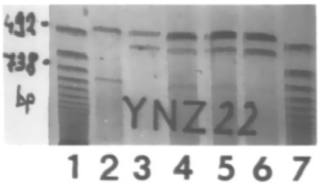

Fig. 2: Amplification of sperm mixtures from 2 individuals in 3 different ratios in comparison to the corresponding blood DNA
lane 1,7: 123 bp ladder (Gibco-BRL, UK)
lane 2 : blood DNA from individual 1
lane 3 : blood DNA from individual 2
lane 4 : semen mixture in 1:1 ratio (25:25ng template DNA)
lane 5 : semen mixture in 1:5 ratio (10:50ng template DNA)
lane 6 : semen mixture in 1:10 ratio (5 :50ng template DNA)

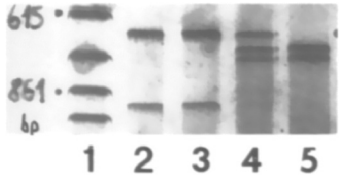

Fig. 3: Amplification of a semen mixture (1:5) from 2 individuals in comparison to the corresponding blood and semen DNA. System: APOB
lane 1: 123 bp ladder (Gibco-BRL, UK)
lane 2: blood DNA from individual 1
lane 3: semen DNA from individual 2
lane 4: semen mixture from individual 1 and 2 (10:50ng template DNA)
lane 5: blood DNA from individual 2

A situation which occurs frequently in practice is that in vaginal smears the concentration of vaginal cells is much greater than the spermatozoa. If a preferential lysis (Gill et al 1985) is carried out, a considerable number of spermatozoa could be lysed in the first step and will therefore be lost. Performing single lysis on the total extract has the disadvantage that the weak component can be suppressed. Therefore for such cases a "mild preferential lysis" was applied. This procedure avoids excess destruction of spermatozoa in the first step and achieves a considerable reduction of female DNA. It is achieved by halving the incubation time and incubation temperature and reduction of the amount of proteinase-K.
After single lysis were the female bands much more intensive than the male because of the unfavourable proportions. After mild preferential lysis the vaginal proportion was distinctly reduced (Fig. 4). The female bands became weaker but now both male bands are visible.

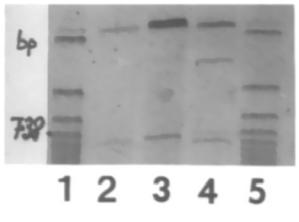

Fig. 4: Amplification of DNA fragments extracted from vaginal swabs in comparison to the female blood DNA. System: YNZ22
lane 1,5 : 123 bp ladder (Gibco-BRL, UK)
lane 2 : female blood DNA
lane 3 : swab extraction, single lysis
lane 4 : swab extraction, mild preferential lysis

A further application of these PCR systems is the analysis of hairs. If hair roots are available there is a good chance of obtaining interpretable results.
One hair root was extracted with Chelex and approximately 5 ng DNA were used for amplification. For both systems (APOB, COL2A1) the expected band patterns were visible (Fig. 5).

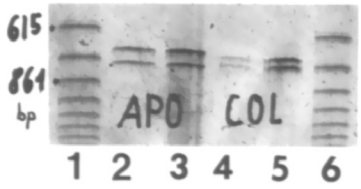

Fig. 5: Chelex extracted hair root DNA in comparison to the corresponding blood DNA using the APO B and COL2A1 systems
lane 1,6 : 123 bp ladder (Gibco-BRL, UK)
lane 2,4 : Chelex extracted hair root (5 ng template DNA)
lane 3,5 : phenol-chlorophorm extracted blood DNA

The amplification of hair shaft DNA only is much more problematical: the blood DNA pattern showed 2 bands, after amplification using 2 hair shafts only 1 of the 2 bands was visible (Fig. 6). Such results can lead to a misinterpretation.

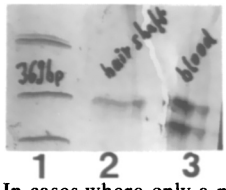

Fig. 6: Comparison between amplified hair shaft DNA and corresponding blood DNA. System: MCT118
lane 1: 123 bp ladder (Gibco-BRL, UK)
lane 2: DNA from 2 hair shafts, extracted after Higuchi (1989)
lane 3: phenol-chloroform extracted blood DNA

In cases where only a minimal amount of DNA is available, coamplification of 2 systems can be useful. Using side-to-side comparison with blood DNA the corresponding bands can be clearly distinguished (Fig. 7).

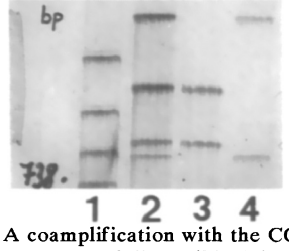

Fig. 7: Coamplification of 2 systems using blood DNA
lane 1: 123 bp ladder (Gibco-BRL, UK)
lane 2: coamplification of the systems MCT118 and YNZ22
lane 3: corresponding DNA fragments in MCT118
lane 4: corresponding DNA fragments in YNZ22

A coamplification with the COL2A1 system and the sex-specific Amelogenin system (Akane et al. 1991) was successfull possible (Fig. 8). Males have 2 bands in defined positions, females only 1 band. Because the ranges do not overlap, these systems can be successfully combined.

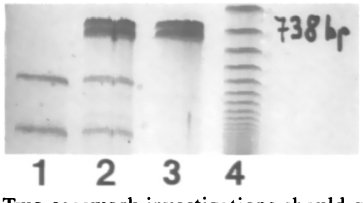

Fig. 8: Coamplification of 2 systems using blood DNA
lane 1: male specific Amelogenin bands
lane 2: coamplification of the Amelogenin and COL2A1 system
lane 3: corresponding COL2A1 bands
lane 4: 123 bp ladder (Gibco-BRL, UK)

Two casework investigations should give an example for the forensic application of the AMPFLP systems: In both cases only highly degraded DNA could be extracted.
The first case involved 2 suspects and 2-year-old cigarette butts (4 butts) were investigated. The band pattern shows that both suspects could be excluded with YNZ22 (Fig. 9) and MCT (not shown).

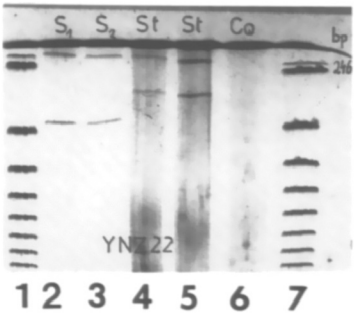

Fig. 9: Amplification of DNA fragments extracted from 2-year old cigarette butts in comparison to the band pattern of 2 excluded suspects
lane 1,7: 123 bp ladder (Gibco-BRL, UK)
lane 2 : blood DNA from suspect 1
lane 3 : blood DNA from suspect 2
lane 4 : DNA extracted from cigarette butts amplified with addition of BSA (200 µg/ml)
lane 5 : DNA extracted from cigarette butts amplified with addition of BSA (400 µg/ml)
lane 6 : negative control, no template DNA was added to the amplification

The last example is a rape case. Clear results could be obtained with 3 AMPFLP systems (Fig. 10; 1 system is shown). The swab extraction was carried out in a 1 step procedure without preferential lysis. In the stain pattern the bands corresponding to the victim and to the suspect could be clearly distinguished. Victim and suspect have 1 common band in the same position.

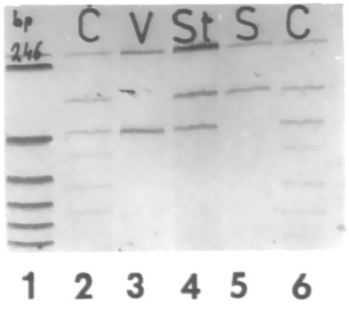

Fig. 10: Amplified DNA fragments in a rape case.
System: YNZ22
lane 1 : 123 bp ladder (Gibco-BRL, UK)
lane 2,6: YNZ22 allele cocktail
lane 3 : victim blood DNA
lane 4 : swab extration, single lysis
lane 5 : suspect blood DNA

To summarise: The AMPFLP systems provide a very promising new generation of methods for forensic haemogenetics which can be applied to solve numerous forensic problems. However one of the prerequisites for the application is the establishment of own data bases and the performance of numerous experimental investigations by each laboratory, so that possible artefacts can be assessed and taken into consideration when interpreting results.

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