# DNA Typing of Fingerprints and Skin Debris: Sensitivity of Capillary Electrophoresis in Forensic Applications using Multiplex PCR

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## ABSTRACT

DNA fingerprinting was performed on fingerprints and skin debris left on hafts and grips of tools, bags and clothes. Different multiplex polymerase chain reaction (PCR) were used allowing simultaneous amplification, detection and analysis of in total fifteen polymorphic Short Tandem Repeat (STR) loci, including the thirteen STR fragments recommended and used by the FBI for the CODIS program. Capillary electrophoresis with automated laser fluorescence detection was used to detect four fluorescent dyes, enabling the use of an internal lane standard within each sample. These results show that DNA fingerprinting can in this way replace the routinely used fingerprint analysis by powder or spray, which in many cases inhibits DNA analysis.

## 1. INTRODUCTION

Short tandem repeat (STR) polymorphisms hold the greatest promise for DNA typing in forensic applications (1, 2). The small size of tetrameric and pentameric STRs facilitates their simultaneous study in a multiplex PCR, in which 2 or more loci are amplified in one reaction from a single DNA sample. Several multiplex reaction kits are already commercially available such as PowerPlex™ and Profiler™. The multiplex PCR can be coupled with the direct detection of amplified products using capillary electrophoresis. This method makes automation of the DNA analysis possible. Automated injection, separation and detection make capillary electrophoresis an efficient technique for sample processing (3). Following the PCR amplification, internal standards can be added to the sample and rapid, reproducible electrophoretic separations can be conducted.

Until now, DNA fingerprinting is routinely used in criminal cases for analysis of blood (stains), saliva, sperm (stains), hair and saliva on cigarette butts, pop cans and bottles. In several crime cases, however, objects and clothes are left at the scene of the crime with at first sight no obvious traces for DNA fingerprinting.

This paper describes the use of DNA fingerprinting methodology on fingerprints and skin debris left on tools (chisel, screwdriver, hammer, etc), clothes (glove, trousers, hat, shirt, etc) and bags. Several crime cases were this way solved in our laboratory.

We have created a multiplex of 4 STR loci (4) and have extended this to 7 STR loci. We also compared the two commercially available multiplex kits PowerPlex™ and Profiler™. In this way, DNA fingerprinting is performed using all loci recommended by the FBI for the CODIS program (5).

## 2. MATERIALS AND METHODS

### *2.1. Samples and Extraction*

Samples for DNA fingerprinting were taken using a sterile scalpel or swab. Fingerprints left on the grips and hafts of tools were wiped off using a sterile swab. All samples were stored at -20°C. DNA was extracted using the slightly modified (6) version of the Chelex extraction method (7, 8). The extracts were stored at -20°C.

### *2.2. PCR amplification*

PCR was performed on a DNA thermal cycler 480 from Perkin Elmer. At first a multiplex of 7 STR loci (CD4, TH01, D21S11, SE33, D16S539, D8S1179 and D18S51) was used. The characteristics of the STR systems used are given in Table 1.

All 7 primers were synthesized by Perkin Elmer. The forward primers were labeled with one of the fluorescent dye markers FAM, HEX or TET. The reverse primers were not labelled.

Of each DNA extract 30 µl was used and the following mixture was added, reaching a final volume of 47.3 µl: 9 pmol of each CD4 primer, 16 pmol of each D21S11 primer, 18 pmol of each TH01 primer, 9 pmol of each SE33 primer, 8 pmol of each D16S539 primer, 8 pmol of each D18S51 primer, 18 pmol of each D8S1179 primer, 21.15 mM of each deoxyribonucleoside triphosphate (dNTP), 9.51 mM Gene Amp® 10 x PCR, 0.23 g/l albumin and 1.90 mM $MgCl_2$. To each reaction 1.5 units of Ampli*Taq* DNA polymerase was added. The polymerase enzyme was only added when the reaction mix had

reached a temperature of 94°C ('hot start' PCR), resulting in a more specific PCR reaction.

The amplification parameters were: 94°C for 2 min, followed by 33 cycles of denaturation for 60 s at 94°C, annealing for 60 s at 58°C and extension for 80 s at 72°C. This was followed by a final elongation step of 5 min at 72°C. At the end of the PCR reaction the temperature was kept at 4°C.

The commercially available multiplex kits, PowerPlex™ and Profiler™, were used according to the manufacturers instructions. The PowerPlex™ kit used was the beta test version 1.2.

1. Powerplex™

The cycling profile of the PowerPlex™ was 95°C for 11 min (initial incubation), 96°C for 2 min, followed by 10 cycles of denaturation for 60 s at 94°C, annealing for 60 s at 60°C and extension for 90 s at 70°C and then by 20 cycles of denaturation for 60 s at 90°C, annealing for 60 s at 60°C and extension for 90 s at 70°C. This was followed by a final elongation step of 30 min at 60°C. At the end of the PCR reaction, the temperature was kept at 4°C.

2. Profiler™

The cycling profile of the Profiler™ was 95°C for 11 min (initial incubation), followed by 28 cycles of denaturation for 60 s at 94°C, annealing for 60 s at 59°C and extension for 60 s at 72°C. This was followed by a final elongation step of 45 min at 60°C. At the end of the PCR reaction the temperature was kept at 25°C.

An amplification blank with all reagents except DNA was included in all experiments. Extraction and purification of DNA, PCR setup in a laminar airflow bench, amplification procedure and analysis of the amplified products were performed in three separate rooms.

2.3. Separation and Detection

The STR loci were analyzed on the ABI Model 310 Genetic Analyzer equipped with an automatic sampler (48 samples). Laser fluorescence-technology was used as detection method.

Capillary electrophoresis was performed on a capillary filled with a non-cross linked polymer, Performance Optimized Polymer 4 (POP-4). With this polymer a capillary with a total length of 47 cm was used and a length to the detector of 36 cm. The i.d. of the capillary was 50 µm (supplied by Perkin Elmer, Foster City, USA). Both at the anode and the cathode side the same buffer was mounted, 1 X Genetic Analyzer Buffer containing 1 mM EDTA. The polymer was injected into the capillary at the anode side using pressure applied by a syringe.

Each STR amplification product (1 µl) from the multiplex of 7 STR loci was mixed with 0.5 µl GeneScan 500 TAMRA internal lane standard and with 12 µl formamide. For the Profiler™, 0.5 µl GeneScan 350 ROX internal lane standard was used and for the PowerPlex™ 0.5 µl CXR labeled internal lane standard instead of the GeneScan 500 TAMRA internal lane standard. These mixtures were then subjected to heat denaturation in the PCR thermocycler for 3 min at 95°C. After cooling on ice, the samples were put in the automatic sampler.

The samples were injected electrocinetically at the cathode side applying a voltage of 15 kV for 10 seconds. Electrophoresis was performed at a voltage of 15 kV for 28 minutes and the capillary was kept at 60°C. Total cycle time for a sample analyzed with capillary electrophoresis was 32 min. After the run of each sample the polymer was removed from the capillary.

Fragment sizes were determined automatically using the GeneScan software employing the method of second order regression to establish a curve of best fit for the internal standard in each lane. This information was used to size all unknown fragments. The capillary electrophoresis used in our laboratory was validated and alleles could be measured within 1 bp difference.

## 3. RESULTS AND DISCUSSION

### *3.1. DNA Extraction*

The Chelex extraction method is routinely used in our laboratory for a wide variety of sample matrices, such as blood, bloodstains on different types of carriers, sperm such as sperm stains on clothing; saliva, e.g. on cigarette butts, pop cans, bottles and stamps; hairs, dirt under fingernails and other biological material used as proof in forensic cases. This method has been validated in our lab as part of a necessary condition of accreditation of forensic labs, and proven to be very reliable and robust. The Chelex extraction method was also successful for clothes, tools and bags.

### *3.2. PCR Optimization*

"Hot start" PCR was used to avoid unspecific amplification leading to unspecific fragments. We amplified 3

STR loci (D16S539, D8S1179 and D18S51) using the same conditions for the multiplex of 4 STR loci (CD4, TH01, D21S11 and SE33) originally used, which we already optimized (4). The fluorescent labels were chosen in order to make the 3 loci compatible with the originally used multiplex of 4 STR loci. We succeeded in creating a multiplex PCR of these 7 STR loci. All primer concentrations were optimized in order to obtain comparable signal intensities for all STR loci.

The concentration of DNA available on clothes, tools and grips of bags is often very low. Therefore the parameters of the PCR amplification were optimized in order to get optimal profiles. For the same reason PCR reactions were sometimes performed with 40 cycles instead of the 33 cycles routinely used in our laboratory. This generally resulted in better profiles. However, sometimes this resulted in more complex DNA profiles, which were more difficult to interpret.

### *3.3. Case study*

The results from a case study are given in tables 2 - 4. The DNA profiles obtained from clothes gave the best results. It was generally possible to obtain a complete DNA profile of all loci without extra amplification. Both commercially available kits gave good results although the intensity of the observed peaks was smaller than in our optimized multiplex PCR. This difference was more seen in the DNA profiles obtained from tools and grips of bags. Here the extra amplification of 40 cycles was generally necessary. It was often seen that the peak(s) for D8S1179 was missed when the DNA profile was weak.

The results obtained by the Profiler™ for tools and bags were better than the results obtained by the Powerplex.™ In both kits a profile for the D7S820 locus was rarely seen. It is necessary to take into consideration that the PowerPlex™ kit used was a beta test version for the ABI 310. An extra amplification could be helpful to obtain better profiles in both cases.

Although single DNA profiles were seen, mixed profiles occurred often. It was necessary to inform the local police departments to be very careful when samples were taken. The results from this study show that the technique used is very sensitive and that it is absolutely necessary to avoid contamination. Problems were encountered in obtaining good DNA fingerprints, when the objects were powdered or sprayed for regular fingerprint analysis. Therefore a systematic study is underway in our laboratory to investigate the influence of different fingerprint methods on DNA typing.

## 4. CONCLUDING REMARKS

It is generally accepted that DNA fingerprinting is a very useful tool in crime solving, not only for blood samples, sperm or saliva. When a sensitive technique such as capillary electrophoresis is used, it is possible to get a profile from fingerprints left on tools and bottles or skin debris left on clothes. In the future it may be possible to replace standard fingerprint analysis by powder or spray, which in many cases inhibits DNA analysis. Therefore it is necessary to have a DNA database which is common and accessible to all forensic laboratories. The computer program CODIS will certainly be of great help to obtain this goal.

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**Table 1**
Characteristics of the STR loci under study

| system | chromosome location | reference | product size (bases) | primers |
|---|---|---|---|---|
| **CD4** | 12p | (9) | 142 - 177 | TTACGCGTTTGGAGTCGCAAGCTGAACTAGCG (forward) **labeled with FAM amidite**<br><br>CCAGGAAGTTGAGGCTGCAGTGAA (reverse) |
| **TH01** | 11p15-15.5 | (1, 10) | 178 - 207 | GTGGGCTGAAAAGCTCCCGATTAT (forward) **labeled with HEX amidite**<br><br>ATTCAAAGGGTATCTGGGCTCTGG (reverse) |
| **D21S11** | 21 | (11) | 172 - 264 | GTGAGTCAATTCCCCAAG (forward) **labeled with FAM amidite**<br><br>GTTGTATTAGTCAATGTTCTCC (reverse) |
| **SE33** | 6 | (12) | 222 - 339 | ACATCTCCCCTACCGCTATA (forward) **labeled with HEX amidite**<br><br>AATCTGGGCGACAAGAGTGA (reverse) |
| **D8S1179** | 8 | (13) | 161 - 201 | TTTTTGTATTTCATGTGTACATTCG (forward) **labeled with TET amidite**<br><br>CGTAGCTATAATTAGTTCATTTTCA (reverse) |
| **D18S51** | 18q21.3 | (13) | 271 - 343 | CAAACCCGACTACCAGCAAC (forward) **labeled with TET amidite**<br><br>GAGCCATGTTCATGCCACTG (reverse) |
| **D16S539** | 16q22-24 | CHLC web site (14) | 141 - 173 | GATCCCAAGCTCTTCCTCTT (forward) **labeled with HEX amidite**<br><br>ACGTTTGTGTGTGCATCTGT (reverse) |

**Table 2**

Results: representative examples in the case study of 7 STR fragments

| | **CLOTHES**[2] | **TOOLS**[3] | **BAGS**[4] |
|---|---|---|---|
| **CD4** | 5 | 5, 10 | 5 |
| **D21S11** | 29, 30 | 28, 32 | 30 |
| **TH01** | 6, 7 | 6 | 7, 9.3 |
| **D18S51** | 13 | 12, 13 | 12, 14 |
| **D8S1179** | 13, 14 | 13 | 12, 13 |
| **D16S539** | 11, 13 | 11, 13 | 13 |
| **SE33**[1] | 255, 255 | 271, 295 | 247, 315 |

**Table 3**

Results Powerplex™ representative examples in the case study

| | **CLOTHES**[2] | **TOOLS**[3] | **BAGS**[4] |
|---|---|---|---|
| **Amelogenin** | X, Y | X, Y | X, Y |
| **vWA** | 16, 18 | 16, 18 | 16, 18 |
| **TH01** | 6, 7 | 6 | 7, 9.3 |
| **TPOX** | 8 | | 10, 11 |
| **CSF1PO** | 11, 12 | 12 | 12 |
| **D7S820** | 11 | | |
| **D5S818** | 11, 12 | 11 | 11 |
| **D13S317** | 11, 13 | | |
| **D16S539** | 11, 13 | 11, 13 | 13 |

**Table 4**

Results Profiler™: representative examples in the case study

| | **CLOTHES**[2] | **TOOLS**[3] | **BAGS**[4] |
|---|---|---|---|
| **Amelogenin** | X, Y | X, Y | X, Y |
| **vWA** | 16, 18 | 16, 18 | 16, 18 |
| **TH01** | 6, 7 | 6 | 7, 9.3 |
| **TPOX** | 8 | 10, 11 | 10, 11 |
| **CSF1PO** | 11, 12 | 12 | 12 |
| **D7S820** | 11 | | |
| **D5S818** | 11, 12 | 11 | 11 |
| **D13S317** | 11, 13 | 8, 12 | 8, 12 |
| **D3S1358** | 14, 17 | 16, 17 | 15, 17 |
| **FGA** | 19, 22.2 | 22 | 23, 24 |

[1] Length of alleles
[2] Gloves
[3] Screwdriver
[4] Grip of bag