

Use of the *GenePrint*[™] CSF1PO, TPOX, TH01 Multiplex System (CTT) for the Analysis of DNA Evidence in a Serial Killer Case

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INTRODUCTION

The amplification of short tandem repeat (STR*) loci using the polymerase chain reaction (PCR) is currently the method of choice in forensic investigations. Use of STR loci can overcome the limitations of VNTR systems, producing reliable and highly discriminating results with 1ng or less of sample material. Casework samples may contain limited amounts of partially degraded DNA as the only evidence that may link a suspect to a crime. In addition, quality assurance standards for forensic testing laboratories request the retention of a portion of the evidence sample for additional testing whenever feasible. The availability of STR multiplex systems makes it possible to meet these criteria and to provide adequate discrimination for the analysis of forensic casework samples.

It must be kept in mind that multiplex reactions are a compromise of ideal conditions. Loss of efficiency of amplification may occur when analyzing poor quality samples and there may be occasions where it is beneficial to use the same loci in monoplex tests to assist interpretation. In this report, the analysis of DNA evidence in a serial killer case is presented as an example of one of the first high publicity court cases where STR multiplex systems were used. Since these events occurred, multiplexing of STRs has been accepted by the European courts in several hundred cases. Currently, such systems are used for the development of criminal intelligence databases or database pilot projects in some European countries. Such databases will eventually include DNA profiles from millions of individuals and from unsolved crimes, and will have the potential to link suspects to crime scenes, and crime scenes to one another.

EVIDENCE OF SERIAL KILLER ACTIVITY

On September 15, 1990, near the city of Prague, the body of a woman was discovered in a trench, partially submerged in water and left in sexual disarray. The identity of the victim was soon established. The cause of death was determined to be the result of ligature strangulation. No relevant biological evidence could be recovered from the victim or the crime scene. The case remained open until Spring 1992.

Between January 1991 and April 1992 the bodies of seven prostitutes were discovered in wooded areas in different parts of Austria. The victims were reported missing between 16 days and 353 days prior to the discovery of their bodies. Thus, in four cases, the remains were either badly decomposed or skeletonized. All cases shared certain characteristics with regard to the cause and manner of death, the pattern of disposal and positioning of the bodies, the state of dress and the clothing and personal belongings left behind at the disposal site and on the bodies. Where the cause of death could be determined, it was found to be ligature strangulation using a piece of the victim's clothing (panty hose or shirt). The bodies were disposed of either naked in sexual disarray or with the clothing partially removed. No relevant biological evidence suitable for DNA analysis could be recovered from any of these cases.

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In June and July 1991, the bodies of three prostitutes were discovered in Los Angeles. All three had been strangled with their own brassieres. The Los Angeles Sheriff's Department and the Los Angeles Police Department established a link between the three murders, based predominantly on the special characteristics of cuttings applied to the brassieres and the knots found in the ligatures. These three cases were unknown to the authorities in Austria and in the Czech Republic until September 1991.

In May 1991, a potential suspect for the murders in Austria was presented to the Austrian authorities by a retired homicide detective. He recalled the murder of an 18-year-old girl who had been strangled with her own brassiere in Germany in 1974. The suspect was a 44-year-old man who had been sentenced to life imprisonment in 1976 for a total of 15 crimes, including the 1974 murder of the German girl. During his imprisonment, he began a career as an author and was able to establish himself as an example of successful rehabilitation. Eventually he was granted parole and was released from jail in May 1990. During the following two years, he was cruising by car through Austria, Germany, Italy and Eastern Europe, directing performances of his theater plays and working as a journalist, writing articles mostly dealing with prostitution.

Because of the unusually high rate of prostitute murders in Austria between January 1991 and April 1992, and because of similarities between some of the murders,

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Figure 1. Head hair recovered from underneath the seat cover of the suspect's car.

there was strong evidence for the activity of a serial killer. In connection with this, Interpol unsolved cases in other European countries were investigated. The Czech authorities provided the unsolved case from Prague. While checking the suspect's alibi the police established that he had been in Prague on the day that the girl was murdered. Similarly, an association between his traveling activities and the disappearance of the victims was established for the Austrian cases.

Furthermore, he had flown to Los Angeles in June and returned in July 1991. While he was in Los Angeles, he managed to get a tour with police officers of areas frequented by prostitutes, under the premise that he worked as a reporter. During his visit to Los Angeles three prostitutes, who had been working the streets near the hotels where he was lodging, were murdered. In the months after his return to Vienna he was under police observation. In February 1992, he flew to Miami, Florida, where he was arrested and subsequently extradited to Austria for trial.

Circumstantial evidence against the suspect was based mainly on the fact that the police were able to establish an association between his traveling activities and the murders of the 11 victims. Certain similarities existed between all the cases and he could not provide alibis for any of them. There were also strong similarities to the murder of the German girl in 1974, for which he had been convicted.

DNA ANALYSIS

In June 1992, investigators recovered several head hairs with attached root or sheath material from underneath the seat cover of the front passenger seat of a BMW that the suspect had driven in September 1990, at the time when the Prague murder took place (Figure 1). He subsequently sold the car and it was eventually scrapped. However, the car seats had been removed and had been sitting in a garage for almost 18 months. The recovered hairs were submitted for microscopic analysis to the crime laboratory of the Scientific Police Institute in Zurich, Switzerland, in July 1992. Three of the hairs were microscopically indistinguishable from those of the Prague homicide victim. In April 1993 – a time when the era of multiplexing STRs had just begun – the Institute of Legal Medicine in Bern, Switzerland, was requested to use DNA analysis to determine if these minute, old hair samples could belong to the Prague victim. In this case, the quantity of DNA available and the typing methods used became critical issues.

DNA was isolated from one centimeter of the root portion from each of the hairs using the phenol/Centricon® 100 (Amicon) method. Nine nanograms of human DNA were extracted from one of the three evidentiary hair samples from the suspect's car. No DNA could be extracted from the other two samples. In June 1993, two nanograms of

DNA from this evidentiary sample were amplified and typed for HLA-DQA1 using the AmpliType® HLA-DQ Alpha Amplification and Typing Kit (Roche Molecular Systems, Inc.). This analysis resulted in a match with the victim's reference head hair samples and a frequency of 1 in 13. In order to conserve as much of the original evidentiary sample as possible for further analysis, the Genomic DNA from the HLA-DQA1 amplification product was purified, recovered and subsequently typed for the five Polymarker loci using the AmpliType® PM PCR Amplification and Typing Kit (Roche Molecular Systems, Inc.). This led to a confirmation of the match and a frequency of 1 in 2,300.

Finally, in April 1994, two nanograms of DNA from each sample were amplified and typed simultaneously at the three STR loci, TH01, TPOX and CSF1PO, using the then newly developed *GenePrint*™ STR Multiplex System (CTT) (Figure 2). In June 1994 the hair was determined to have come from a female. This was accomplished by amplifying one nanogram of DNA at the X-Y homologous amelogenin locus. Adding the *GenePrint*™ STR typing and the sex typing a match was again obtained and the power of discrimination increased dramatically to approximately 1 in 2.1 million women.

In order to confirm the identity of the individual contributing the hair sample recovered from the suspect's car by different means, a reverse paternity analysis was performed. It demonstrated that the donor of the evidentiary sample could not be excluded as the child of the Prague homicide victim's mother and father.

THE TRIAL

The trial was held in Austria in 1994. There is no equivalent to the Frye standard in the Austrian court system; however, the DNA methodology, including the underlying population genetics statistics, applied in this case was considered so well accepted and noncontroversial that the judge ruled it admissible without holding a pretrial hearing. The DNA evidence was presented by a qualified expert who had participated in the analysis, in an extensive one and a half-day hearing. This included the education of the jury by addressing the principles of DNA analysis and PCR. Photographs of all typing results were mounted on large cardboard posters for courtroom demonstration.

Although the field of multiplexing STRs had just begun, the jury learned that a reliable, powerful and widely accepted scientific procedure had demonstrated that a piece of

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biological evidence found in the suspect's car was highly likely to have come from one of the victims.

The defense chose two ways to attack the analysis. The first argument was that although the DNA test results were carried out properly there were gaps in the chain of custody. This argument was refuted by demonstrating an exact protocol of the chain of custody of the evidence by all participating laboratories and investigators. For the DNA part, it was explained that the known and questioned samples had been received, extracted, amplified and typed independently.

The second argument was over the statistical basis of using a small population sample to calculate the frequency of occurrence of the DNA profile. However, despite the size of the population sample, tests for independence had been carried out that have

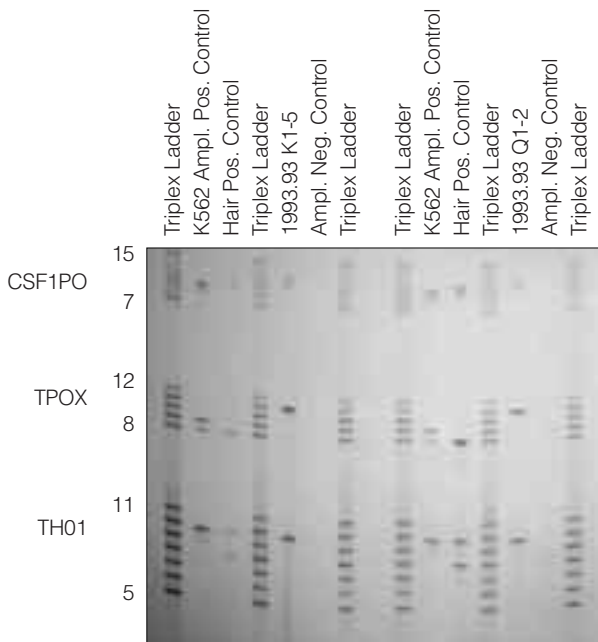


Figure 2. Silver-stained DNA profiles of the STR loci TH01, TPOX and CSF1PO amplified using the *GenePrint*™ CTT Multiplex System. The DNA typing results from a reference head hair from the victim (1993.93 K1-5) are the same as those from the recovered head hair from the car seat (1993.93 Q1-2). The STR ladder alleles are labeled to the left of the image. The numbers indicate the sizes of the smallest and largest alleles in each allelic ladder.

sufficient power to detect departures from expectations that would affect estimates of the likelihood of the occurrence of a DNA profile. It was shown that the population sample used in this case could yield reliable estimates of the frequency of a multiple PCR-based DNA profile. Furthermore, frequencies for different population samples were provided, demonstrating that this particular nine-marker profile was very rare in all populations.

THE VERDICT

The suspect was sentenced to life imprisonment for the murder of nine women, including the Prague homicide, from which the DNA evidence had been analyzed. DNA evidence played a central role in the trial and established with a high degree of certainty that this particular victim had been in contact with the suspect's car.

The verdict was read to the suspect at 9 pm; six hours later he hanged himself in his cell. As the suspect had made an appeal against the conviction before he committed suicide, under Austrian law he must be considered not guilty of these crimes and theoretically the cases will remain unsolved.

THE POWER OF STR MULTIPLEX SYSTEMS

Since only nine nanograms of human DNA could be recovered from the hair, sample size and power of discrimination of the applied tests were important issues in this case. This case also demonstrates the power

of STR multiplex systems in investigations where the quality and quantity of DNA is a critical issue (i.e., in a range below 10ng). Using STR multiplex amplification systems, typing of specific regions of ten genetic markers was possible while consuming only five nanograms of the sample DNA. Nearly half of the sample extract was retained for additional testing by the defense, an option that was ultimately not chosen.

CONCLUSION

Multiplex STR amplification systems are reliable, easily applied systems that have now been used widely in European court cases. The inclusion of allelic ladders with each system provides a rapid and accurate method of allele determination that is easy to present to a jury without necessitating a detailed explanation of the underlying scientific principles. The development of both manual silver stain and automated fluorescence detection methods for the same STR systems provides universal application across laboratories with different levels of funding and sophistication.

The community has recognized that it is vital to implement and ensure the highest quality assurance standards for all forensic testing laboratories presenting cases like this in court. If such standards are met, there is no doubt that the analysis of DNA polymorphism using multiplex systems represents an extremely useful, practical and reliable tool for typing biological evidence recovered at crime scenes.

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The development and use of STR loci is covered by U.S. Patent No. 5,364,759 assigned to Baylor College of Medicine, Houston, Texas. Rights have been licensed to Promega Corporation for all applications. Most applications have been licensed on an exclusive basis. U.S. Pat. No. 5,599,666 has been issued to Promega Corporation for allelic ladders for the loci CSF1PO, F13A01, FESFPS, LPL and vWA.

Use of the *GenePrint*[™] STR System requires performance of the polymerase chain reaction (PCR), which is the subject of European Pat. Nos. 201,184 and 200,362, and U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-La Roche. Purchase of the *GenePrint*[™] STR System does not include or provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of the *GenePrint*[™] STR System may, therefore, be required to obtain a patent license, depending on the country in which the system is used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.