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LCN DNA typing from touched objects

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Abstract. A married beautiful woman received, in different slots, in her home two envelops containing pornographic photos and indecent proposals from an anonymous persistent suitor together with underclothes showing semen stains. DNA typing showed that both stamps were licked by the same male individual who even was the donor of semen traces. The comparison with DNA profiles obtained from two marking pens generally used by a suspect and a comb belonging to him showed a compatibility between profiles. This casework is a further confirmation that it is possible to type LCN DNA with very good results if an appropriate collection and analysis of biological material is performed. © 2006 Published by Elsevier B.V.

Keywords: LCN DNA; STRs; Identifiler kit

1. Introduction

A married beautiful woman received, in different slots, in her home two envelops containing photos and indecent proposals from an anonymous persistent suitor. The woman sent the material above to our laboratory for latent prints development and for searching biological traces for DNA typing. While latent prints research gave negative results, we were able to find by amylase test some saliva traces on the stamps sufficient to perform DNA analysis.

Since the husband of the woman suspected a colleague, after some weeks from the analysis above, he brought us two marking pens (one red and the other one black) that they generally employed at the workplace, for trying to perform DNA typing from any eventual sweat/skin residuals found on them so as to compare all DNA profiles obtained.

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As a further control, was used a comb left by the suspect in the trash. While we were analyzing samples, the woman received a parcel containing expensive underwear that she immediately sent us to search for any eventual biological fluid. By a special lamp "Polilight" at variable wavelength, we found on the bra and the tanga some semen stains.

2. Materials and methods

In order to minimize the possibility of contamination, all extractions were set up in a Gelman laminar flow cabinet in a dedicated laboratory.

DNA analysis has been performed on saliva traces found on the stamps, sweat traces found on the pens, epithelial cells found on the comb, semen stains found on the underclothes, and the oral swab belonging to the husband. DNA extraction from saliva on the stamps and from semen stains was performed incubating at 56 °C for 30 min, a fragment of each stamp and the oral swab in about 200 μl of "Chelex" containing 5 μl of Proteinase K, 10 mg/ml. Then samples were boiled for 8 min [1].

Material found over the marking pens and over the comb after their observation by the special lamp Polilight was taken swabbing gently each item by sterile swabs soaked by PBS 1X. Swabs were directly incubated at 56 °C for 3 h in about 200 μl of "Instant Gene Matrix" (Biorad) containing 5 μl of Proteinase K 10 mg/ml and 5 μl DTT 1 M and then boiled for 8 min [2]. Samples were then purified and concentrated by ultrafiltration in centricon 100.

All extracts were quantified by the Quantifiler Human DNA Quantification kit (Applied Biosystems) using a 7300 Real-Time PCR System kit and following the manufacturer's protocol [3].

The amount of DNA extracted from the traces on the marking pens and from the cells on the comb was less than 100 pg. Amplification was carried out in a laboratory different from the one dedicated to the extraction, so that amplified products never entered the extraction laboratory. STRs amplification was carried out by Gene Amp 9700 and 2400 thermal cyclers with the "AmpFlSTR Identifiler" kit (Applied Biosystem) that uses a five-dye fluorescent system for automated DNA fragment analysis and co-amplifies the repeat regions of 15STRs repeat loci (D19S433, D3S1358, D5S8118, D8S1179, vWA, TH01, D13S317, D21S11, TPOX, FGA, D7S820, D16S539, D18S51, CSF1PO, D2S1338). A segment of the X-Y homologus gene Amelogenin is also amplified, for gender diagnosis [4]. For samples with LCN DNA, the number of PCR cycles was increased to 35 using a reduced reaction volume. PCR tests were performed two times. For the other samples, amplification was performed following the manufacturer's protocol. Different positive and negative controls were enclosed during the amplification steps so as to verify the possibility of any contamination from a laboratory source because of the high sensibility of the procedure [5].

Amplified products were analyzed by capillary electrophoresis on a ABI PRISM 310 Genetic Analyzer (Applied Biosystems) employing ABI softwares (DATA Collection, GeneScan Analysis, Genotyper Fragment Analysis). For fragment length determination of the PCR products, the internal lane DNA standard LIZ 500 (Applied Biosystems) and the external allelic ladder were used for calibration.

3. Results and discussion

DNA typing of all samples showed that

1. both stamps were licked by the same male individual;
2. the red marking pen showed a mixed full DNA profile;
3. the black marking pen showed a partial DNA profile;
4. the mixed DNA profile was compatible with a mixture between the partial DNA profile found on the black pen and the DNA profile of the husband;
5. all DNA profiles were compatible with the one of the men who licked the stamps;
6. semen stains on the underclothes were from the man who licked stamps;
7. cells found on the comb (belonging to the suspect) had the same DNA profile of saliva found on the stamps.

Statistical calculation of genotype distribution were used in the frequencies of the population to which analyzed samples were belonging [6,7]. It showed that stamps DNA profile had a frequency of 2.54×10^{20} DNA analysis confirmed the hypothesis: the colleague of the husband was the perpetrator of the crime.

This casework is a further confirmation that it is possible to type LCN DNA with very good results if an appropriate collection and analysis of biological material is performed.

4. Conclusions

Low copy number (LCN) DNA profiling is a technique sensitive enough to analyze just a few cells. The extreme sensitivity of the method requires many precautions either during the analysis to reduce the risk of contamination from personnel within the laboratory itself and between samples than during the interpretation of data since the possibility to observe artefacts or high stutters (due to the increased number of cycles), allelic drop out (preferential amplification). So the replication of PCR tests, wherever possible, is suggested for verifying the profiles obtained.

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