Cyt-*b* analysis and hair comparison in serial robbery cases

A. Berti, A. Virgili, G. Zignale, M. Serafini, G. Lago *

Sezione Biologia, Reparto Carabinieri Investigazioni Scientifiche, via Aurelia 511, 00165 Rome, Italy

1. Introduction

Biological specimens from animals can be found in criminal investigations for different reasons.

We report a serial robbery case which occurred in October 2000 in Rome. In 1 month, over six robberies with the same modus operandi were perpetrated. All victims reported that the robber was a man with a dog; moreover, the robber used the dog to frighten his victims.

Police found many dog hairs in all of the victim's cars and immediately submitted them to our laboratory. To confirm that the hairs found belonged to a dog, we extracted the DNA from the hair root and perform cyt-*b* analysis for species identification. We amplified a 290-bp cyt-*b* gene and sequenced it by the BigDye terminator method. The sample was analyzed on an ABI 310 Genetic Analyzer and the sequence was submitted to the BLAST DNA sequences database. The Database search confirmed that the hair belonged to a dog. After genetic analysis, we analyzed the hair microscopically and compared it to different hair samples belonging to several races of dog as reference samples. One of the reference hair samples perfectly matched, in size, color and morphology, the hair found at the crime scenes. Results were immediately transmitted to the Public Prosecutor, and the robber was soon arrested.

2. Materials and methods

DNA extraction from dog hair roots was carried out essentially as described in Sambrook et al. [2]. The samples were digested at 37 °C overnight in 200 μl extraction buffer (10 mM Tris–HCl pH 8, 100 mM NaCl, 10 mM EDTA, 2% SDS, 39 mM DTT) in

* Corresponding author. Tel.: +39-6-6639-4644/4665; fax: +39-6-6639-4737.
E-mail address: gplago@tiscalinet.it (G. Lago).

a single tube with 15 μl of Proteinase K (20 mg/ml). The DNA was phenol−chloroform extracted and ethanol precipitated. Finally, the samples were resuspended in 20 μl of sterile de-ionized water. Cyt-*b* analysis was carried out essentially as described in Bataille et al. [1]. Two sets of primers were used in a single reaction mix: cyt-*b* forward and reverse primers and HV1 L15997 and H16236 primers.

The PCR mix was as follows: 0.4 M of cyt-*b* primers, 0.6 M of D-Loop HV1 primers, 1.25 U Taq polymerase, 0.2 M each dNTP, 1.5 mM MgCl2, 50 mM KCl and 10 mM Tris−HCl pH 8.3 in 25 μl. Electrophoresis on agarose gel allows screening for human (two bands, 309 and 259 bp) or animal (one band, 309 bp) nature. Both strands of the cyt-*b* PCR products were sequenced by the dideoxynucleotide method of Sanger using the
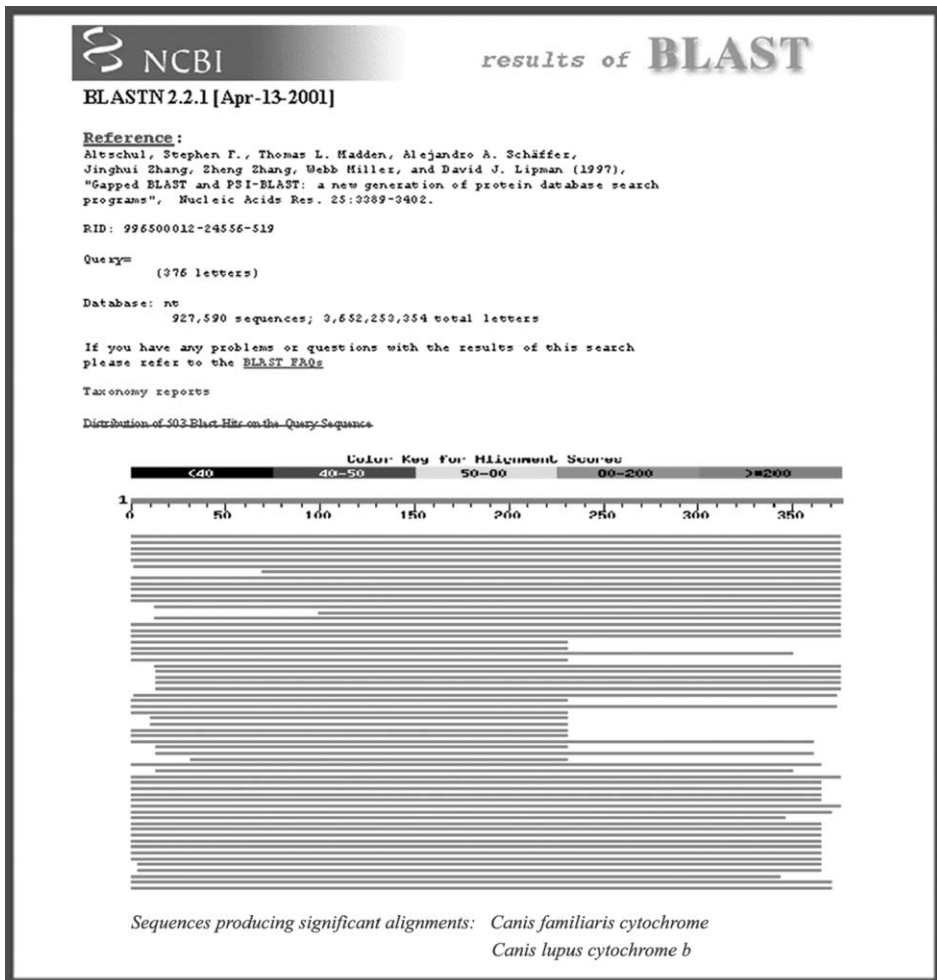

Picture 1. Response of search on BLAST sequences DNA database.

BigDye terminator sequencing kit from Applied Biosystems, with the primers described above, followed by detection on an ABI 310 instruments (Applied Biosystems). The sequence was submitted to the BLAST sequences DNA database.

http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST). The database search confirmed that the hair belonged to a dog (Picture 1). Dog hair comparison was carried out by microscopy analysis on a LEICA DMR at $40 \times$ magnification with white light.

3. Results and discussion

Species identification in forensic analysis is not always easy. When we have a biological sample such as a hair root, species identification by immunological methods is impossible. In these cases, cyt-*b* analysis is recommended. We used this technique in a serial robbery case which occurred in October 2000 in Rome. At the crime scene, police
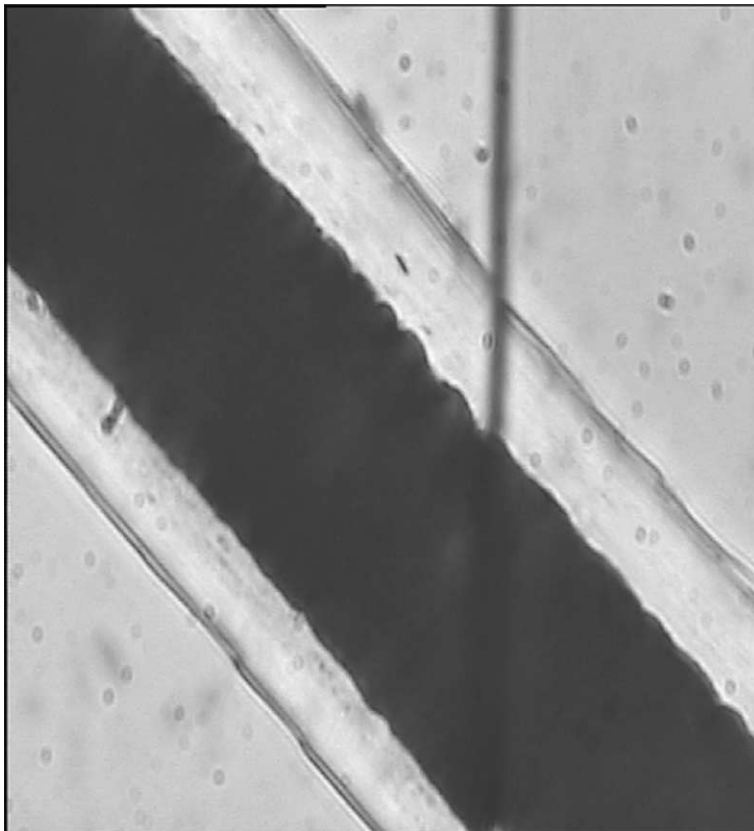
Picture 2. Microscopical dog hair image.

found many dog hairs that we analyzed by applying the cyt-*b* test (Picture 2) followed by microscopy analysis (Picture 3). Both techniques confirmed the nature of the hair. Moreover, comparison with different reference samples allowed us to identify the race
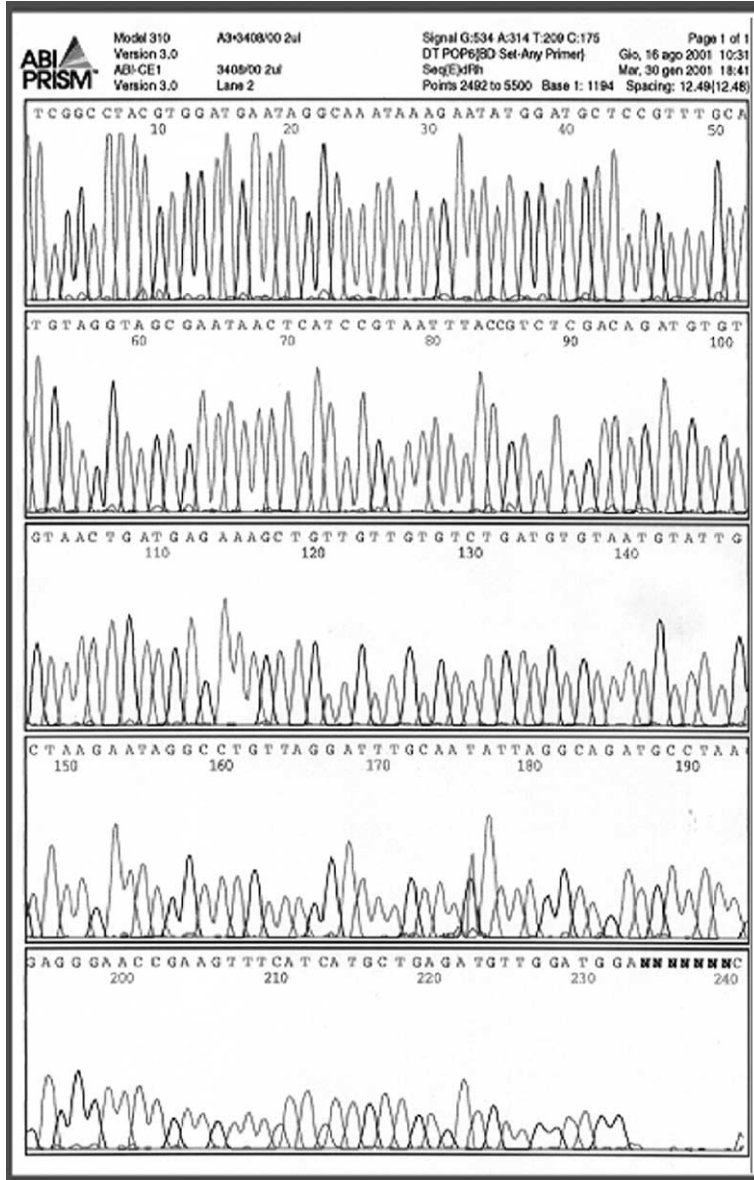
Picture 3.

of the dog. The results were immediately transmitted to the Public Prosecutor and the robber was soon arrested.

References

[1] M. Bataille, K. Crainic, M. Leterreux, M. Durigon, P. Mazancourt, Forensic Sci. Int. 99 (3) (1999), 165–170.
[2] J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press, NY, 1989.