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# Acta Medicinae Legalis et Socialis

IMPRENSA DA UNIVERSIDADE DE COIMBRA
COIMBRA UNIVERSITY PRESS

ACADEMIA INTERNATIONALIS MEDICINAE LEGALIS

COIMBRA 2010

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# SPECIES IDENTIFICATION FROM GENETIC MATERIAL WITH CYTOCHROME b

**Abstract:** Cytochrome b gene has a genetic content preserved between animals and it is used especially for the vertebrate's identification. In the forensic context it is specially used to determine the origin of non human samples. The sequencing strategy that was implemented in our laboratory, BigDey/BetterBuffer/XTerminator, allowed to reduce time procedures, improve quality of data and reduce significantly the cost per reaction comparing to other described methodologies. Using the program BLAST the obtained sequence is aligned and compared with sequences of the cytochrome b gene registered in a database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The aim of this study is the identification of several vertebrate animals to allow the resolution of eventual forensic cases in a shorter time than traditional methodologies and with lower costs.

## Introduction

The nucleotide sequence of the cytochrome b (cytb) region of the mitochondrial DNA (mtDNA) holds specific information about diverse animal species. The analysis of the sequence of the cytb has been used in the identification of species in phylogenetic and forensic genetics fields. There are cases in the forensic context (traffic accidents involving animals, illegal traffic or hunter of animals, human attack perpetrated by animals, among others) in which identification of species is necessary for the case to be solved (Branicki *et al.*, 2003).

The aim of this study is the identification of several vertebrate animals to allow the resolution of eventual forensic cases in a shorter time than traditional methodologies and with lower costs.

## Materials and Methods

Blood samples were obtained from diverse mammals and birds. DNA was extracted using Chelex®100 method (Walsh *et al.*, 1991). PCR amplification was performed with primers L14816 and H15173 (Parson *et al.*, 2000) and chemistry of QIAGEN® Multiplex PCR Master Mix in a final volume of 25 µl. Thermocycling conditions

were performed in a GeneAmp® PCR system 2700 (Applied Biosystems), followed the Parson *et al.*, (2000) protocol. The cycle sequencing was performed using the ABI Prism® BigDye® Terminator v.1.1 Cycle Sequence Kit (Applied Biosystems); BetterBuffer (Microzone Limited, Sussek, UK) has been incorporated into the sequencing procedure. Before DNA analysis a simple bead purification method (XTerminator) was made, to remove the unincorporated BigDye terminators, unnecessary salts, and unused diluent buffer. The sequences were analysed in the sequencer 3130 – Genetic Analyser (ABI PRISM®) with the ABI DNA Sequencing Analysis Software v.5.2 and the SeqScape® Software v.2.5. Species were identified with BLAST 2.2.19+ (Smith *et al.*, 1996, Zhang *et al.*, 2000).

## Results

Using the cytochrome b gene and Blast tool we were able to identify multiple species including *Homo sapiens, Canis familiaris*, *Felis catus*, *Ovis aries* and *Turdus viscivorus*. Figures 1, 2 and 3 show the steps that allow identifying samples species (in this case a bird sample).

## Discussion

The sequencing strategy BigDye/BetterBuffer/XTerminator compared with others (for example dRhodamine/ ethanolic precipitation) allow to reduce time procedures, improve quality of data and reduce significantly the cost per reaction.

## Conclusions

Results show that it is possible to identify multiple species with this low cost and faster methodology.

## References


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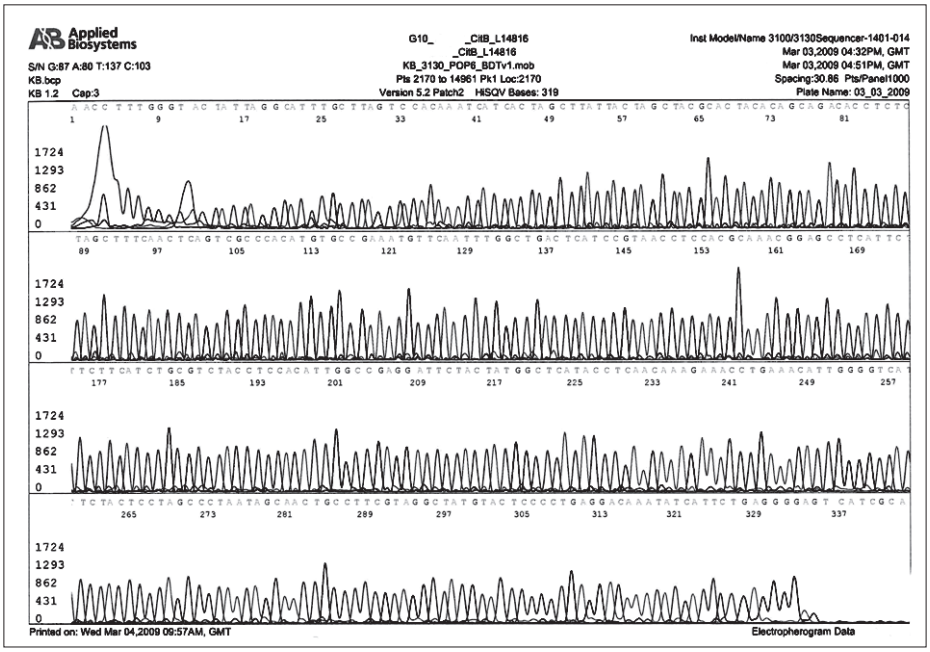


Figure 1 – Electropherogram sequence of *Turdus viscivorus* cytochrome b gene.

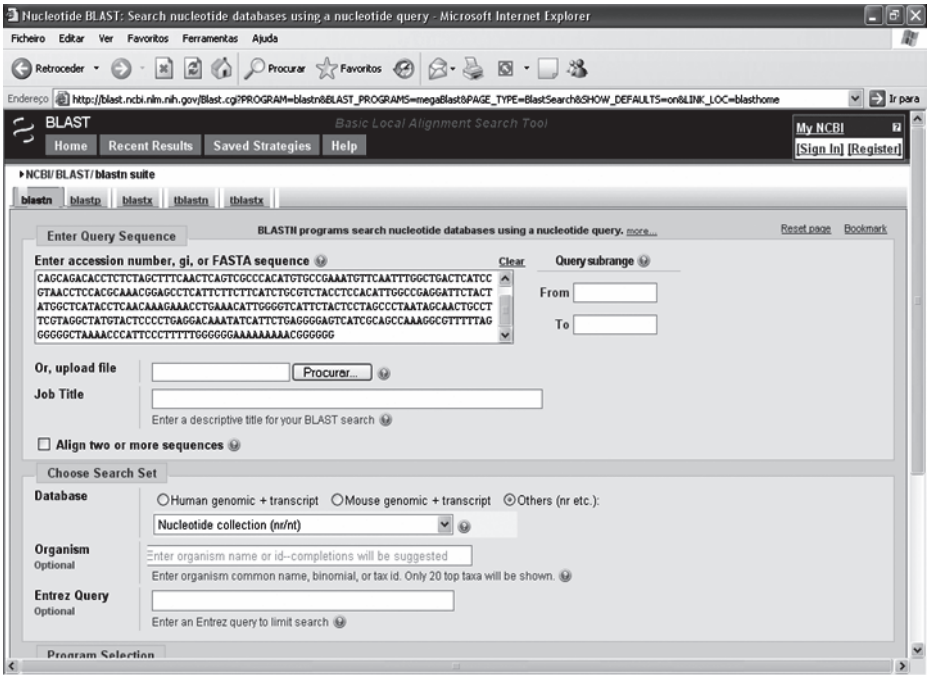


Figure 2 – Input on to Blast Web page of the nucleotide sequence as a query against all the public sequence databases [http://www.ncbi.nlm.nih.gov/BLAST/].

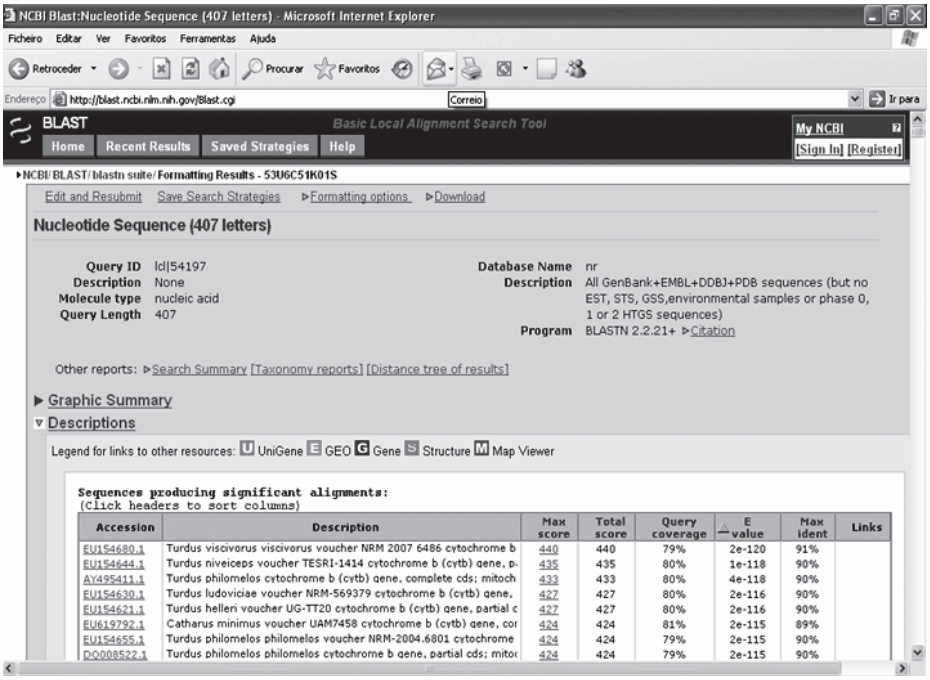


Figure 3 – Results of the search performed on the NCBI database and servers.