

MITOCHONDRIAL DNA BARCODING AS A TOOL FOR THE GENETIC TRACEABILITY OF FISHERY DERIVATIVES

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DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a gene of known function and position in the genome. The core idea of this technique is that nucleotide variation for short pieces of DNA can be mostly found between species and only to very low extents among organisms within species. DNA markers suitable for genetic traceability purposes usually belong to the mitochondrial genome because of its haploid nature, maternal inheritance, and multiple copies in the cell. COI (Cytochrome oxidase subunit I) was originally used as specific mitochondrial gene for DNA barcoding: a 648 nucleotide long sequence was selected near to the 5' end of the gene with two conserved flanking sites in most animal groups where universal primers were then designed. These primers supplied very reliable results in all taxa tested so far and they also enabled the recovery of gene polymorphisms for most animal phyla. Besides, the evolution of COI is rapid enough, because of the high incidence of base substitutions in third-position nucleotides, to allow the discrimination of closely related species and sometimes phylogeographic groups within a single species. Several studies have now established that sequence diversity in this portion of COI provides strong resolution at the species level for several animal groups including birds and fishes.

The aim of our research is to apply the DNA barcoding as an helpful strategy for genetic traceability of marine species and their food derivatives, with particular reference to three different taxonomic groups: fishes, molluscs and shellfishes. According to the criticisms recently provided by scientific community supporting the theory that a single gene may not be sufficient to univocally identify a species, in addition to the Cytochrome oxidase subunit I (COI) we have selected other two mitochondrial genes encoding for Cytochrome b (Cyt b) and ribosomal RNA small subunit (16S-RNA). After the selection of species on the basis of their economic relevance, the experimental steps adopted for the *in silico* analyses were the following: i) retrieval of mitochondrial sequences from NCBI nucleotide databases for each of the selected genes; ii) removal of redundant and unreliable entries, and editing of sequences; iii) evaluation of intra- and inter-specific polymorphisms (SNPs and In/Dels) by means of multiple alignments; iv) design of forward and reverse primers on highly conserved regions of the consensus sequences for each gene and group of species belonging to the same family. We are now performing serial *in vivo* analyses using genomic DNA samples isolated from commercial food products containing one or more fish, mollusc and shellfish species. A number of DNA samples from pure species for each family were also included as reference standards. The experimental steps currently adopted for the identification of species are the following: i) amplification of the target sequences using the specific primer pairs; ii) subcloning and sequencing of the PCR products; iii) Blast analysis against the non-redundant nucleotide databases using each of the sequences as query; iv) computation of the substitution

matrices and identity indices for species identification. The research is in progress with the main goal of setting a multilocus genetic traceability system, assessing the organism taxonomic identity and verifying the food product label information.