DETERMINATION OF FISH SPECIES USING POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM (PCR-RFLP)

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PENGHARGAAN

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ABSTRACT

Seven types of fish sold for human consumption were subjected to DNA extraction using Qiagen DNeasy® Tissue Kit. The genomic DNA of only five fish samples was successfully extracted. The extracted DNA was then subjected to PCR amplification targeting the 359 base pair (bp) mitochondrial cytochrome b (cyt b) gene. A pair of universal primers cyt *b1* and cyt *b2* was used which consistently amplified a fragment of the cyt b gene of the fish samples. All fish samples except *Megalaspis cordyla* were successfully amplified.

The 359 bp amplicons of PCR assay of fish samples were then subjected to RFLP using four different restriction enzymes, namely *Bfa* I, *Hinf* I, *Msp* I and *Mbo* II. None of the fish samples was fragmented by *Bfa* I. *Polydactylus pleibeius* and *Rastrelliger kanagurta* were also not being cleaved into fragments when their DNA were subjected to digestion with Msp I. However, the 359 bp of *Pampus argenteus* and *Trachinotus bailonii* were further fragmented into two when they were digested with *Msp* I. The same results were obtained when the 359 base pairs of all fish samples were digested with *Hinf* I and *Mbo* II. The DNAs were cleaved into one to two fragments. The fragments generated have different sizes i.e. in a range of 104 bp to 300 bp. The results obtained were unique to the fish types. Three different REs (*Hinf* I, *Msp* I and *Mbo* II) were found to be sufficient in determining the fish species thus indicating that the PCR-RFLP analysis of cyt b represents a rapid, simple and promising method for differentiation of fish species.