A New Method for Rapid DNA Extraction from *Artemia* (Branchiopoda, Crustacea)

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Abstract**—***Artemia* is one of the most conspicuous invertebrates associated with aquaculture. It can be considered as a model organism, offering numerous advantages for comprehensive and multidisciplinary studies using morphologic or molecular methods. Since DNA extraction is an important step of any molecular experiment, a new and a rapid method of DNA extraction from adult *Artemia* was described in this study. Besides, the efficiency of this technique was compared with two widely used alternative techniques, namely Chelex® 100 resin and SDS-chloroform methods. Data analysis revealed that the new method is the easiest and the most cost effective method among the other methods which allows a quick and efficient extraction of DNA from the adult animal.

Keywords**—**APD, *Artemia*, DNA extraction, Molecular experiments

I. INTRODUCTION

IT has been years that the brine shrimp *Artemia*, mainly because of its versatile use and its nutritional value, is considered as one of the most widely used live foods in larviculture [1]. In addition this animal is also a known model for genetic and evolutionary studies [2]. Molecular experiments on *Artemia*, not only as scientific approaches for evolutionary analysis, but also as tools in the commercial industry of *Artemia* hold promise to be widely used methods [3]-[4].The isolation and purification of DNA from biological samples for subsequent molecular analysis is generally the most important and undervalued step in many biological and biomedical applications [5]. DNA extraction comprises three basic steps: The first step is cell breaking, commonly referred to as cell disruption, in order to expose the DNA within, using different methods such as grinding, sonicating, thermal shock or chemical reagents.

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The second step is removing membrane lipids by adding a detergent. The last step is the precipitation of DNA by an alcohol, usually ethanol or isopropanol. Since DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation. This step also removes alcohol-soluble salts, PCR inhibitors (e.g. enzymes or ions). A refinement of the technique includes adding a chelating agent to sequester divalent cations such as Mg^{2+} and Ca^{2+}. This stops DNAase enzymes for further DNA degradation. Moreover, cellular and histone proteins that are bound to DNA can be removed prior to its precipitation, for instance either by adding protease or sodium and ammonium acetate. The objective of these procedures is to achieve a yield of the highest purity as quick as possible.

Contamination during DNA extraction mainly due to the repetition of sample transferring between tubes is the main defect of conventional methods. Therefore it is necessary to set separate vials for each individual sample and to apply a long extraction protocol. Although the traditional DNA purification methods such as organic extraction and chelex extraction have demonstrated good ability to remove the interfering contaminants, they are not amenable to the trends in genetic analysis towards miniaturization and automation because most of these purification procedures require a large amount of DNA samples, reagent volumes, multiple steps and time-consuming labour [6]. In this paper, a new surfactant (Ammonium Pyrrolidin Dithiocarbamate, APD complex) was developed for effective DNA extraction under an easy protocol.

Elimination of materials by surfactants is one of the most favorable methods of purification, due to their excellent binding ability for a variety of biomolecules and ions. Chelating resins or surfactants have been used in ion-exchange columns, trace metal removal, metal analysis and water testing in environmental and agricultural laboratories. In clinical applications and biomedical research, these materials can be used to remove or assay cations in whole blood or urine, to remove contaminants from buffers and stock solutions, and to prepare samples for nuclear magnetic resonance spectroscopy. Recently the solid phases modified with immobilized organic compounds have been attracting great interest because of the high enrichment capacity and operational simplicity for solid phase extraction of metal ions [7]-[8]. It is well understood that, in an acidic medium, sodium dodecyl sulfate (SDS) as an anionic surfactant is sorbent on the alumina to form surfactant aggregates. In fact, hydrophobic chelating agents can