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RESEARCH PAPER

Optimizing DNA Extraction Method for Archived Otoliths

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Abstract

The DNA from the archive collections is very important for fisheries biologists, administrators, systematics and conservation biologists. Archival tissues allow access to the genetic information required to resolve the link between past and present. However, effective protocols for obtaining DNA from otolith and bone are still insufficient, time consuming and inefficient.

The aim of this study was to develop an extraction protocol for otholits samples based on NucleoSpin TissueXS, Macherey-Nagel commercial kit and perform a quantitative comparison with the extraction protocol based on EDTA-SDS.

Keywords: Archived otoliths, DNA extraction, EDTA-SDS.

Introduction

Archived otoliths offer valuable sources of material for temporal genetic analysis. These sources however often are limited, fragile and easily damaged. Furthermore, DNA extraction from the archived otoliths is generally difficult when compared to those that have been collected recently. Therefore, it has critical importance to develop species-specific combined methods for DNA extraction from achieved otoliths without damaging the physical structure of the otolith.

Otoliths represent a rather limited source of DNA since a maximum of two sagittal otoliths can be collected and archived. Low copy number of template DNA in combination with degradation after years of storage can result in potential DNA contamination, poor amplification success, (large) allelic dropout and false alleles. (Pompanon *et al*., 2005). The use of short fragment markers in combination with high laboratory standards may prevent these artefacts and is needed to guarantee reliable genetic results (Jakobsdottir *et al*., 2006).

The objective of this study was twofold: (i) to develop an optimized DNA extraction protocol (commonly used commercial DNA extraction kit) for archived otoliths based on NucleoSpin TissueXS, Macherey-Nagel commercial kit and (ii) to compare it to the protocol based on EDTA-SDS with the aim to assess DNA quantification

Materials and Methods

In the first protocol, a commercial kit was used. In the second protocol, we developed an extraction method in order to reduce damage to otoliths by modifying EDTA and SDS-based lysis solution. Later on, total DNA was obtained by using standard phenol-chloroform method (Taggart *et al*., 1992).

Total of 30 pairs of archived *Mullus barbatus* otoliths collected in 1989, 1995, 2007 and 2015. They were all collected from Black Sea cost and all samples stored individually in envelope at room temperature (Figure 1). Archived DNA is particularly prone to genotyping errors due to i) its degraded nature increases the risks for allele dropout and null alleles and ii) potential contamination from exogenous DNA. To address these issues, otoliths sample preparation and counter cross-contamination procedures followed the published protocol of Vanek *et.al.* (2009). Otoliths are limited edition, also they do not belong to the same year. The otoliths were belonging to the Central Fisheries Research Institute (CFRI) Trabzon, Turkey.

DNA Extraction

The comparison reported here in used 70-80 mg of fine otholits powder for all extractions. Both NucleoSpin and EDTA-SDS extraction protocols were followed without the decalcification step. To avoid contamination of archived otoliths samples, all

Figure 1. Otoliths and envelopes for sampling and storage from 1989 and from 2015.

DNA extractions, DNA quantifications and subsequent preparations of PCR reactions, were completed under a laminar flow where PCR products were analyzed.

In the First Protocol, Nucleospin Tissue, Macherey-Nagel Commercial Kit Extraction Protocol

Either 80 µL of T1 buffer, 8 µL of proteinase K (20 mg/mL) were added to 70-80 mg of fine otholits powder and the sample was incubated for approximately 18 h at 55°C overnight with shaking at 250 rpm. Then 80 µL of B3 buffer was added and incubated for 5 min at 70°C. The sample was centrifuged at 11,000 x g for 1 min and 80 µL of ethanol (96–100 %) and vortex vigorously. For each sample, a NucleoSpin® Tissue Column was placed into a Collection Tube. We applied the sample to the column. Centrifuge for 1 min at 11,000 x g. Discard Collection Tube with flow-through and placed the column in a new Collection Tube. 50 µL Buffer B5 was added to the NucleoSpin® Tissue XSColumn. Centrifuged for 1 min at 11,000 x g. It was not necessary to discard the flow-through(1 wash). 50 µL Buffer B5 was added to the NucleoSpin® Tissue XS Column. Centrifuge for 2 min at 11,000 x g. Discarded Collection Tube with flow-through (2 wash). The NucleoSpin® TissueXS Column was placed in a new 1.5 mL microcentrifuge tube and 20 µL Buffer BE was applied to the center of the silica membrane of the column. Centrifuge for 1 min at 11,000 x g. Total processing time was approximately 20 h.

In the Second Protocol Based on Edta-Sds Extraction Protocol

Each set of otoliths was incubated at 55°C overnight with shaking at 250 rpm in 500 µL of digestion buffer (100 mmol_L^{-1} Tris–HCl, 100 mmol_L^{-1} NaCl; 1 mmol_L^{-1} EDTA, 0.5% SDS, pH was adjusted to 8.0 and total volume 1000 µL). DNA extraction by standard phenol–chloroform procedures described in Taggart *et.al*. (1992) was followed. The reagents SDS and EDTA are included to digest the lipids in the membranes and to reduce the effects of the DNases during digestion (Sambrook & Russel 2001). Total processing time was approximately 23 h.

PCR Amplification

A forward primer, H1478 5'-TGACTGCAGAGGGTGACGGGCGGTGTGT–3' and a reverse primer, L1091 5'-AAAAAGCTTCAAACTGGGATTAGATACCCCA CTAT-3' were used for the PCR amplification of the 12SrRNA gene (Kocher *et al.*,1989). Amplifications were performed in a GeneAmp PCR System Veriti Thermal Cycler (Applied Biosystems) using the QIAGEN Multiplex PCR Kit. The reaction volume of 20 µL contained 1 µL of template DNA, 10 µL QIAGEN Multiplex PCR Master Mix, 0.5 µL of 5.0 µM forward and reverse primer solutions (according to prior optimization) and 8 µL ultrapurewater. Thirty cycles of amplifications were performed with denaturation 95°C for 2 min, primer annealing at 61°C for 12S rRNA, for 1 min, and primer extension at 72°C for 1 min followed by a final extension at 72°C for 10 min. The PCR products were detected and their size was estimated by electrophoresis of 4 mL of each amplification mixture in 1% agarose gels in 1% Trisborate-EDTA with known molecular weight standards of λDNA/HindIII marker (Promega). The gels were stained with 0.5 mg/ mL ethidium bromide. The PCR products were stored at -20°C until further analysis.

Results

In the present study, we optimised the procedures for extraction of DNA from reduced amounts of exhumed bones. The method used here was to obtain reliable DNA profiles, minimize the chance of contamination and reduce the time and material required. The proposed protocol provides an

alternative option for DNA extraction when small bone fragments such as otoliths are present.

As a result of this study; a significantly higher DNA yield was observed for historical otoliths treated with Protocol 2 modified by Ruggeri *et al*. (2016) in comparison to Protocol 1 (NucleoSpin TissueXS, Magery-Nagel). Although DNA yield was generally low, the PCR amplification success rate of contemporary otolith samples were high for the full range of fragments sizes in both the Protocol 1 and 2 (Figure 2 and Figure 3). The PCR amplification success rate of the historical DNA samples was extremely lower than the those of recent samples. This difference is consistent with many studies reporting a reduced amplification when archived samples are used as DNA source (Leonard, 2008). Although DNA degradation from archived otoliths can increase with time through biological, physical and chemical factors affecting DNA quality, our study did not show a significant difference in amplification success between the 1989 and 2015 samples (Figure 3).

Discussions

Given that various factors affect DNA quality, DNA degradation in archived otoliths increases over time. (Wandeler *et al*., 2007). Despite the availability

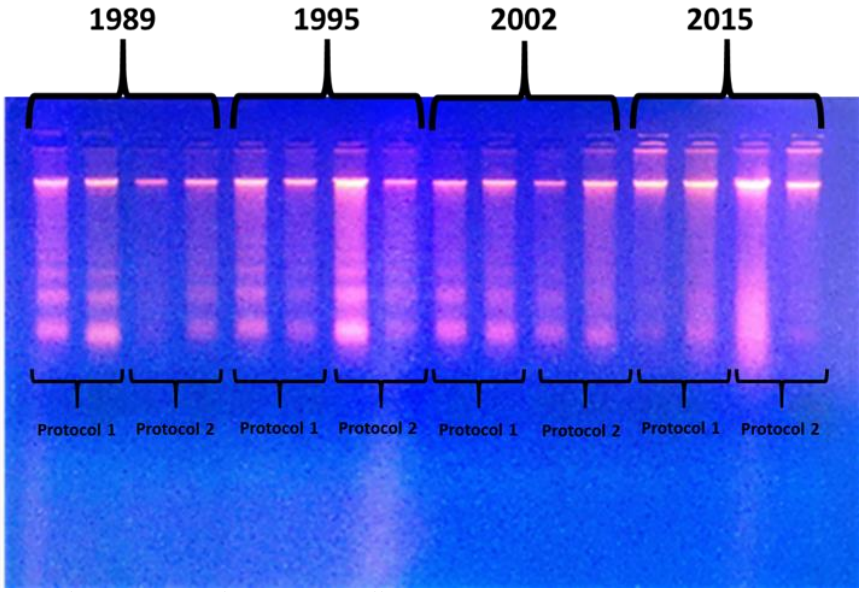

Figure 2. DNA products of the extractions of otholits using different extraction protocol.

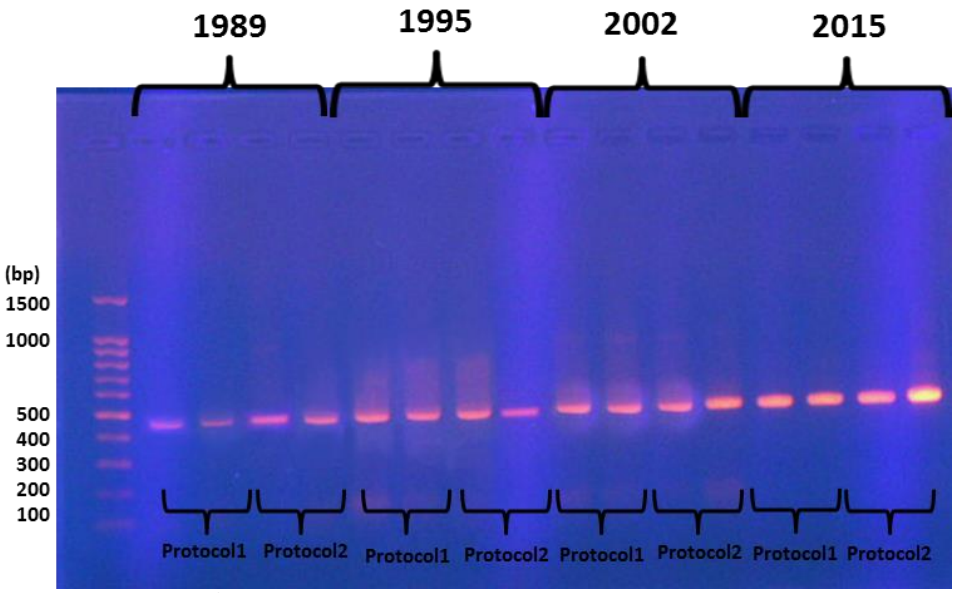

Figure 3. Agarose jel electrophoresis of the PCR product obtained in both protocols.

of all tests from otoliths from the same year, our data do not suggest a higher amplification success in the 2015 sample compared to 1989. Moreover, although all samples were not stored under the same conditions, no effect was noticed on the otolith structure during 26 years of storage. The PCR amplification success rate of the historical DNA samples was found to be lower than that of fresh samples. Existing literature provides several reports on reduced amplification success from archived samples when used as DNA template (Leonard, 2008). Although DNA degradation from archived otoliths can increase with time through biological, physical and chemical factors affecting DNA quality, our study did not show any significant difference in amplification success between the 1989 and 2015 samples.

We did a study on DNA extraction using archived otoliths collections. These collections are a record of the past and they provide links to the past. If materials are not properly archived, it cannot be known that what is lost. As a result, we recommend that one should be careful when choosing an appropriate DNA extraction protocol to avoid damage to the otolith structure. This applies to all work carried out when working with archived samples. High-quality DNA extraction without damaging the otolith structure will open up many new possibilities to better understand the temporal analysis of fish stocks. The proposed protocol provides an alternative option for DNA extraction when small bone fragments such as otolith are present.

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