Steinbrenner Laborsysteme GmbH

magtivio

MagSi-DNA Animal

Application Note | DNA extraction from fish fins for genetic testing by aquaculture laboratories

Introduction

Genomics is key to the development and management of aquaculture. The benefits from genetic improvements may include better growth, resistance to disease or robustness in diverse farming environments. It is of major importance for aquaculture to reveal the genetic basis of performance and production traits, to use such information for genetic enhancement programs, and to monitor the quality of its produce by genomic analysis.

MagSi-DNA Animal allows for a fast and cost-effective extraction of DNA from fresh water and marine fish fins. This application note provides an extensive quantity and quality analysis of DNA extracted from rainbow trout (*Oncorhynchus mykiss*), Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmon salar*) and Japanese amberjack (*Seriola quinqueradiata*), showing the suitability for use in genomic applications (e.g. genotyping by PCR or DNA sequencing).

Materials and methods

Fresh fish were obtained from a local wholesale market. Fish clips were sampled and stored frozen at -20°C until use. After thawing, pieces of 10, 20 and 40 mg of fin tissue were added to 400 µL Lysis Buffer TS and 20 µL Proteinase K (20 mg/mL) for lysis. Lysis was performed with shaking (1000 rpm) at 56°C for 3 hours in a ThermoMixer® C (Eppendorf®) according to standard kit instructions. After briefly spinning down undigested tissue, 300 µL sample lysate was used as input for DNA purification on the PurePrep 96 Nucleic Acid Purification System with a final elution volume of 150 µL.

DNA concentrations of the eluates were measured with the Qubit™ dsDNA BR Assay Kit and purity was assessed by UV-VIS with the NanoDrop™ One according to manufacturer's instructions (Thermo Scientific™) The quality of the DNA samples was evaluated by qPCR on the AriaMx Real-Time PCR system (Agilent) with universal primers targeting the mitochondrial cytochrome oxidase subunit I gene (cox1). DNA samples were diluted 1:10 and 2 µL of each diluted sample was used as template in a total reaction volume of 20 µL (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme).

Results

DNA yields for the different sample amounts used from rainbow trout, Atlantic cod, Atlantic salmon and Japanese amberjack fins are presented in Fig. 1. There is a proportional increase in DNA concentration with increasing amount of input sample, showing the efficiency and tolerance of the extraction procedure for different sample amounts.

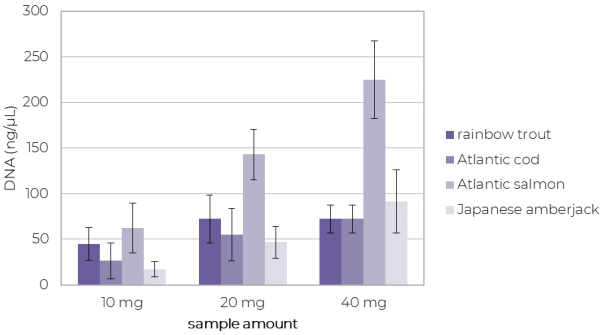

Figure 1. *DNA concentrations obtained with the Qubit™ dsDNA BR Assay Kit. The different input amounts (10, 20 and 40 mg) resulted in a DNA yield ranging from 16 to 225 ng/µL. The data presented are mean values (n= 8, ±1 SD).*

DNA purity obtained from different sample input amounts of rainbow trout, Atlantic cod, Atlantic salmon and Japanese amberjack are shown below in Fig.2. All A260/A280 purity ratio's are ≥1.88 and all A260/A230 ratio's are ≥1.90, indicating highly pure DNA without contamination with proteins or salts. Different input amounts (10 and 40 mg) resulted in equal purity ratios (not shown).

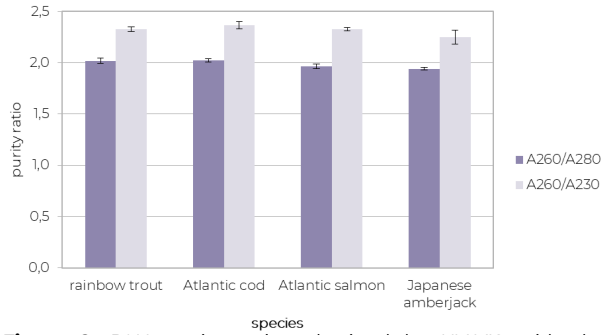

Figure 2. *DNA purity ratios obtained by UV-VIS with the NanoDrop One. The data presented are mean values of DNA extractions from 20 mg sample (n= 8, ±1 SD).*

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The **PCR results** showed an expected decrease of 1 Ct when using a double amount of input sample, demonstrating the suitability for varying sample amounts (Fig. 3).

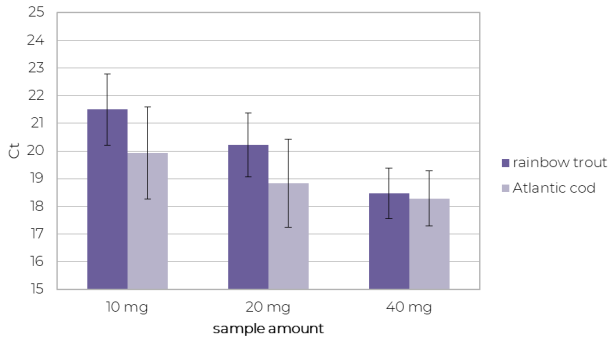

Figure 3. *Ct values obtained from DNA extracted from trout and cod fin by PCR using primers targeting the mitochondrial cytochrome oxidase subunit I gene (cox1). DNA samples were 1:10 diluted before PCR. Ct values between 18 and 22 are reported by the AriaMx Real-Time PCR system (10, 20, 40 mg). The data presented are mean values (n= 8, ±1 SD).*

Conclusion

The data obtained demonstrates that DNA can be successfully extracted from rainbow trout, Atlantic cod, Atlantic salmon and Japanese amberjack.

The obtained DNA concentrations are proportional to the sample input and amounts from 10 to 40 mg can be used without affecting the extraction efficiency. All DNA samples have high purity and PCR results show that the extracted DNA is suitable for genotyping or other applications involving PCR amplication.

It can be concluded that **MagSi-DNA Animal** provides a suitable extraction method for DNA extraction from marine and fresh water fish for PCR and sequencing applications in aquaculture laboratories. Highly standardized sampling procedures are not required as the DNA extraction efficiency is not affected by typical variations in tissue amounts. The extraction protocol can be carried out with minimal equipment requirements, and is easily automated using a magnetic particle processor or liquid handling workstations. In case only low DNA quantities are needed (e.g. SNP genotyping), the processing time can be significantly shortened by decreasing lysis time.

Literature

- *Product Manual MagSi-DNA Animal, PM0023-005, magtivio B.V.*
- *User Guide Qubit™ 1X dsDNA BR Assay, MAN0019617, ThermoFisher Scientific*
- *NanoDrop One UG, 269-309102, ThermoFisher Scientific*
- *Ward et al., 2005: R.D. Ward, T.S. Zemlak, B.H. Innes, et al. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London Series B Biological Sciences, 360 (2005), pp. 1847-1857*

PurePrep 96 Nucleic Acid Purification System

Ordering information

Art. No.	Description	Amount
MDKT00150096	MagSi-DNA Animal	96 preps
MDKT00150960	MagSi-DNA Animal	10 x 96 preps
AS00001	PurePrep 96 Nucleic Acid Purification System	1 unit
MDPL00200050	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	50 pcs/pack
MDPL00190060	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	60 pcs/pack
MDPL00210060	96 well Tip-Comb for KingFisher™/PurePrep 96	60 pcs/pack

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