Steinbrenner Laborsysteme GmbH

magtivio

MagSi-DNA Animal

Application Note | DNA extraction from molluscs for marker-assisted breeding

Introduction

Bivalves, such as cockles, oysters and mussels, are mollusc species that are farmed in extensive aquaculture worldwide. The industrialized aquaculture applies selective breeding of molluscs assisted by genetic markers to overcome disease outbreaks and adverse effects of climate change while improving commercially important traits (e.g. growth and meat yield).

However, these genetic tools can only be used after successful DNA extraction. **MagSi-DNA Animal** allows for a fast and cost-effective extraction of DNA from a wide variety of mollusc samples. This application note provides an extensive quantity and quality analysis of DNA extracted from blue mussels (*Mytilus edilus*), Pacific oysters (*Crassostrea gigas*) and common cockles (*Cerastoderma edule*), using various tissues to show the suitability for use in genetic studies (e.g. genotyping by PCR or DNA sequencing).

Materials and methods

Fresh molluscs (blue mussels, Pacific oysters and common cockles) were obtained from a local wholesale market. Gill, mantel, and adductor muscle tissue samples were collected from each species and 30 mg was transferred into a 96 DeepWell plate already containing Lysis Buffer TS, proteinase K and RNase A for lysis. Lysis was performed at 56° C and 1000 rpm in a Thermomixer C (Eppendorf) for one hour. Afterwards, lysed samples were centrifuged for 15 minutes (6000 x g) to pellet contaminants and sample debris, and 300 µL sample lysate was transferred for use as input for DNA purification on the PurePrep 96 System with a final elution volume of 150 µL.

DNA concentration and purity of the eluates were measured by UV-VIS with the NanoDrop™ One according to manufacturer's instructions (Thermo Scientific™). The absence of inhibitors was evaluated by qPCR on the AriaMx Real-Time PCR system (Agilent) with primers targeting the 18s rRNA gene. From the undiluted (mussel and oyster) and 1:100 diluted (cockle) DNA, 2 µL was used in a total PCR reaction volume of 20 µL (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme).

Results

Yield

DNA yields for gill, mantel and adductor muscle tissues from mussels, oysters and cockles are presented in Fig 1. Yields are highly dependent on the tissue type, resulting in DNA concentrations ranging from 45 ng/µL (adductor muscle) to 262 ng/µL (gills).

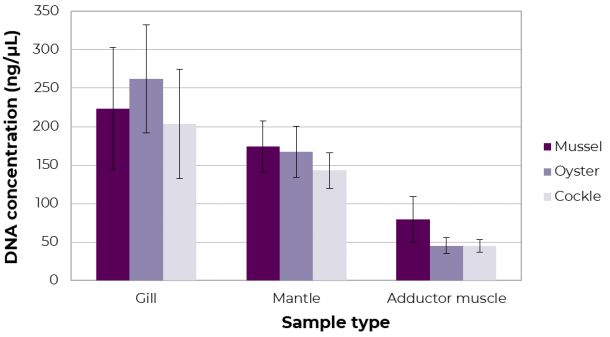

Figure 1. *DNA concentrations after extraction from different mollusc tissues measured with the NanoDrop One. The data are presented as mean (n=3, ± 1 SD).*

Purity

DNA purity ratios of genomic DNA isolated from mussels, oysters and cockles are shown in Fig 2. All A260/A280 purity ratios are ≥ 1.8, indicating good quality DNA.

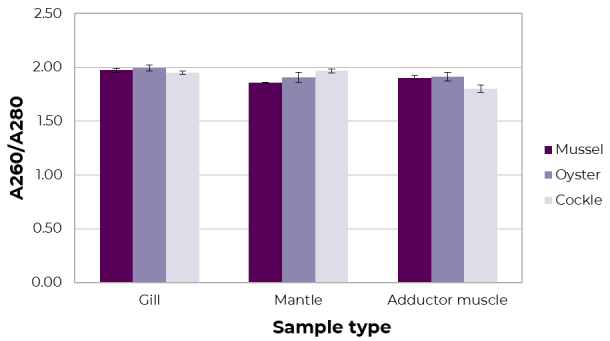

Figure 2. *DNA purity ratios for mussel, oyster and cockle tissues obtained by UV-VIS with the NanoDrop One. High purity ratios are measured for all tissues. The data are presented as mean (n=3, ± 1 SD).*

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PCR

PCR results from different mussel, oyster and cockle tissues are presented in Fig 3. The reported Ct values range from 25 to 30, demonstrating the suitability for extraction and amplification of different mollusc tissues.

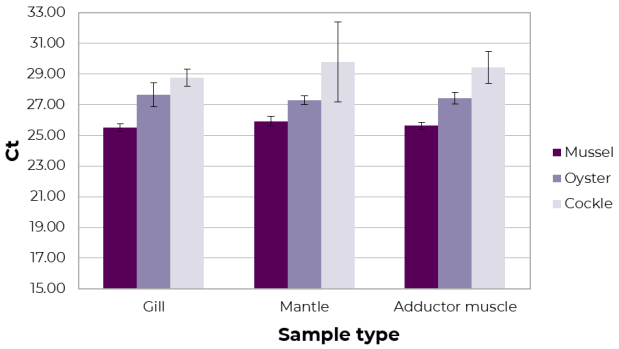

Figure 3. *Ct values obtained from extracted mussel, oyster and cockle DNA by PCR using primers targeting the 18s rRNA gene. DNA samples were undiluted (mussel and oyster) or 1:100 diluted (cockle) before PCR. Data are presented as mean (n=3, ± 1 SD).*

Conclusion and discussion

The data demonstrates that DNA can be successfully extracted from blue mussels, Pacific oysters and common cockles. The extraction protocol can be carried out with minimal equipment requirements, and is easily automated using a magnetic particle processor or liquid handling workstations. Depending on the readout and required information, several tissues can be used such as gill, mantel and adductor muscle tissue. All samples generated DNA with high purity ratio and PCR compatibility. Therefore, it can be concluded that MagSi-DNA Animal provides a suitable extraction method for DNA extraction from molluscs, generating pure DNA that can be used in genetic studies.

Literature

- *Product Manual MagSi-DNA Animal, magtivio B.V.*
- *NanoDrop One UG, 269-309102, ThermoFisher Scientific*
- *Hollenbeck and Johnston, 2018. Genomic Tools and Selective Breeding in Molluscs. Front Genet. 18;9:253.*
- *Jang et al., 2011. Selectively enhanced expression of prophenoloxidase activating enzyme 1 (PPAE1) at a bacteria clearance site in the white shrimp, Litopenaeus vannamei. BMC Immunology 2011 12:70.*

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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