Steinbrenner Laborsysteme GmbH

magtivio

MagSi-DNA Animal

Application Note | DNA extraction from shrimp samples for genomics-assisted breeding

Introduction

Penaeid shrimps are a family of marine crustaceans which contain many species of economic importance, of which whiteleg shrimp (*Litopenaeus vannamei*) and black tiger shrimps (*Penaeus monodon*) are the most widely cultured species in the world. During the last decades, there was a strong increase in cultured shrimp productivity thanks to the genomic improvement of aquaculture species. Genomic selection does not only enhance the growth and the quality of the shrimps but has also the potential to improve disease resistance and environmental robustness.

MagSi-DNA Animal allows for a fast and cost-effective extraction of DNA from aquaculture samples. In this application note, the quality of DNA extraction from marine crustaceans is demonstrated using various tissues of black tiger shrimp that can be collected by non-lethal sampling. The extracted DNA samples were subjected to an extensive DNA quantity and quality analysis, showing the suitability for use in genomic applications (e.g. genotyping by PCR or DNA sequencing).

Materials and methods

Frozen black tiger shrimps were obtained from a local wholesale market and stored at -20°C until DNA extraction. After thawing, antenna's, pereiopods (walking legs), pleopods (swimming legs, whole and ~2 mm top part), and uropods (tails) were collected into a 96-deepwell extraction plate containing 400 µL Lysis Buffer TS, 20 µL Proteinase K (20 mg/mL) and 10 µL RNase A (10 mg/mL). Samples were lysed overnight at 56°C with shaking (1000 rpm) in a ThermoMixer® C (Eppendorf®). After briefly spinning down undigested exoskeleton (chitin-based) and tissue, 300 µL sample lysate was used as input for DNA purification on the PurePrep 96 Nucleic Acid Purification System (final elution volume of 100 µL).

Concentrations of the extracted genomic DNA were quantified by the Qubit™ dsDNA BR Assay Kit and purity was measured using the Nanodrop™ One according to manufacturer's instructions (Thermo Scientific™). PCR reactions were done on the AriaMx Real-Time PCR system (Agilent) with universal primers targeting the cytochrome b gene. DNA samples were diluted 1:10 and 2 µL was used as template in 20 µL total reaction volume (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme).

Results

DNA yields for different sample types are presented in Fig. 1. For all samples, the DNA concentration is higher than 20 ng/µL, showing the efficiency of the extraction procedure for different shrimp parts. DNA extraction from pereiopods results in the highest DNA concentration.

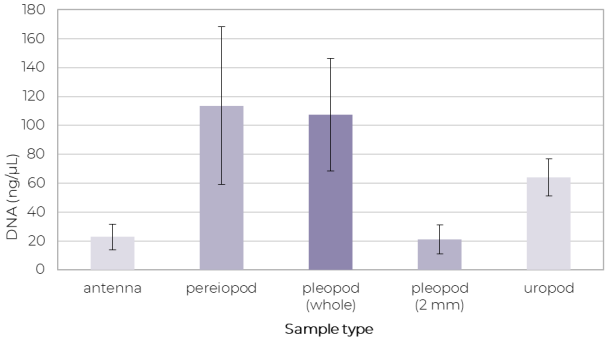

Figure 1. *DNA concentrations obtained with the Qubit™ dsDNA BR Assay Kit. The different shrimp parts resulted in a DNA yield ranging from 21 to 115 ng/µL. The data are presented as mean (n=8, ±1 SD).*

DNA purity ratios for the different sample types are presented in Fig. 2. All A260/A280 purity ratio's are ≥1.8 and all A260/A230 ratios are ≥1.5, indicating highly pure DNA without protein or salt contamination.

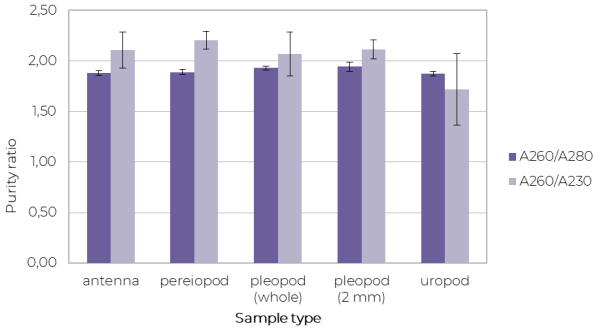

Figure 2. *DNA purity ratio's for different parts of shrimp obtained by UV-VIS with the NanoDrop™ One. High purity ratios are measured for the extracted DNA from all shrimp parts. The data are presented as mean (n=8, ±1 SD).*

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PCR results from different sample types are presented in Fig. 3. Ct values were reported for all samples and ranged from 17 to 27, demonstrating the suitability for different shrimp parts.

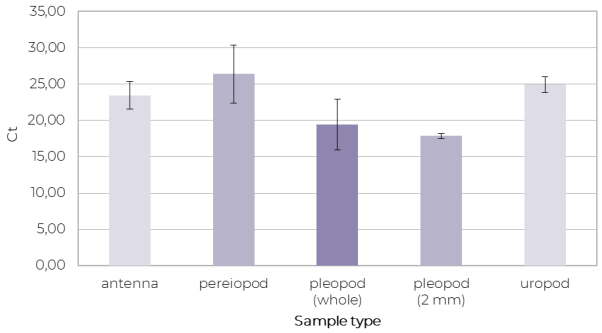

Figure 3. *Ct values for different shrimp parts. Ct values range from 17 to 27. The data are presented as mean (n=8, ±1 SD)*

Conclusion & Discussion

The data obtained demonstrates that highly pure DNA can be successfully extracted from all tested shrimp tissues with **MagSi-DNA Animal.** Differences in obtained DNA concentrations may be related to variations in sample size/amount and chemical exposure to the lysis buffer, but all concentrations are suitable for PCR analysis. It can be concluded that **MagSi-DNA Animal** is suitable for DNA extraction from shrimp samples for genomic testing (PCR or sequencing) in aquaculture laboratories.

For this study, no mechanical disruption was included but for genomic assays requiring more DNA or targeting genes with low copy numbers, samples could be homogenized (e.g. by bead-beating). Entire shrimp parts such as antennas or pleopods as used in this study exceed the typical specified sample amount for most DNA extraction kits, which could lead to PCR inhibition. For this reason laboratories may require a labor-intensive resampling step to obtain smaller parts prior to DNA extraction. The tests for this application note were purposely done on whole shrimp parts to show the extraction quality without such resampling, providing a more efficient workflow for the user.

The extraction protocol of **MagSi-DNA Animal** can be carried out with minimal equipment requirements, and is easily automated using a magnetic particle processor and/or liquid handling workstation. 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*
- *Shekbar et al., 2020. The genomics of the Farmed Shrimp: Current Status and Application, Reviews in Fisheries Science & Aquaculture, 29:4, 654-665*
- *Zenger et al., 2019. Genomic Selection in Aquaculture: Application, Limitations and Opportunities with Special Reference to Marine Shrimp and Pearl Oysters. Front Genet 9:693*

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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