MagSi-DNA Animal

Application Note | DNA extraction from edible insects

Introduction

Insects are of increasing interest for use as animal feed, for human consumption, and in waste management. They have been identified as a promising source of protein in animal feed due to their high nutritional value, easy availability and sustainability compared to traditional sources such as beef or chicken. In addition to animal feed, insects are also valuable for human food production as the expanding global population demands for alternative sources of animal-derived proteins with good nutritional value. Several insect species, including ***Acheta domesticus*** (house cricket) and ***Tenebrio molitor*** (yellow mealworm), are accepted for food applications and human consumption in the EU. While crickets can only be sold as a powder ingredient, mealworm larvae are on the market in dried, frozen, paste and powder formats. In this way, they can be incorporated in standard products such as pasta, bread and burgers.

Large-scale insect breeding asks for genomic selection to obtain high-performance insect lines. Here, we demonstrate the application of the **MagSi-DNA Animal** kit for both DNA and RNA extraction from insects for downstream analysis, such as genotyping by PCR or DNA sequencing. As an example, this application note describes the automated purification of DNA and RNA from house crickets (*Acheta domesticus*) and mealworm larvae (*Tenebrio molitor*).

Materials and methods

DNA and RNA were extracted from *Acheta domesticus* and *Tenebrio molitor* larvae. Live insects were acquired from an online reptile feed store (Reptiel Totaal, Buchten, The Netherlands). Insects were frozen and stored at -20°C until use. DNA and RNA were extracted from *A. domesticus* head (~10 mg), jumping legs (~10 mg), abdomen (~30 mg), and from *T. molitor* larvae head-thorax (~20 mg) and abdominal segments (~20 mg, tail end). The tissues were homogenized by mechanical disruption (8 mm stainless steel beads, 5x 1 min at 1500 rpm) in the 2010 Geno/Grinder® (SPEX® SamplePrep). After homogenization, 400 µL Lysis Buffer TS and 20 µL Proteinase K were added and samples were incubated at 56°C for 30 minutes (ThermoMixer C, Eppendorf).

Disrupted and lysed samples were centrifuged to pellet cell debris, and 300 µL sample lysate was used as input for automated DNA and RNA purification on the PurePrep 96 Nucleic Acid Purification System with a final elution volume of 150 µL .

Concentrations of DNA and RNA in the extracts were assessed by Qubit Fluorometric Quantification (dsDNA BR Assay and RNA BR Assay Kit) according to manufacturer instructions. The NanoDrop™ One (ThermoFisher Scientific) was used to determine the purity of the samples. DNA quality was evaluated by Real-Time PCR on the AriaMx Real-Time PCR system (Agilent) with universal primers targeting the 16S ribosomal RNA region on the mitochondrial DNA, 2 µL template DNA was used in a total reaction volume of 20 µL (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme).

Results

Yield

DNA and RNA concentrations obtained from *A. domesticus* and *T. molitor* sections are presented in Figure 1. The nucleic acid concentrations are highly dependent on the insect species and sample input, ranging from 6 to 85 ng/µL. Highest DNA and RNA concentrations were obtained from the *A. domesticus* abdominal sections.

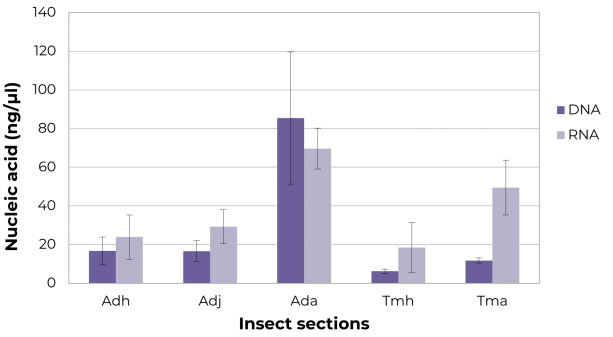

Figure 1. *Nucleic acid concentrations from A. domesticus and T. molitor sections, assessed with the Qubit™ dsDNA BR Assay Kit and Qubit™ RNA BR Assay Kit. The data are presented as mean (n=3, ±1 SD). Adh, A. domesticus head; Adj, A. domesticus jumping legs; Ada, A. domesticus abdomen; Tmh, T. molitor head-thorax; Tma, T. molitor abdomen.*

magtivio B.V.

Daelderweg 9
6361 HK Nuth | The Netherlands

Tel.: +31 (0)45 208 4810
Fax: +31 (0)45 208 4817
www.magtivio.com
info@magtivio.com

Purity

All data exhibited A260/A280 purity ratios between 1.92 and 2.02, which is in the expected range for pure RNA (Table 1). The corresponding A260/A230 purity ratios are >1.8, indicating high quality, uncontaminated DNA, RNA.

Table 1. *DNA purity ratios (UV-VIS) with the NanoDrop™ One.*

	A260/A280	A260/A230
A. domesticus head	2.01	2.30
A. domesticus jumping legs	2.02	2.28
A. domesticus abdomen	2.00	2.28
T. molitor larvae head-thorax	1.92	1.88
T. molitor larvae abdomen	2.01	1.90

Data are presented as mean (n=3)

PCR compatibility

Real-Time PCR results from *A. domesticus* and *T. molitor* DNA are shown in Figure 2. Ct values between 11 and 23 were reported. The expected increase in Ct value (~3.3 per 10-fold dilution) was observed for all sample types, indicating the absence of PCR inhibition.

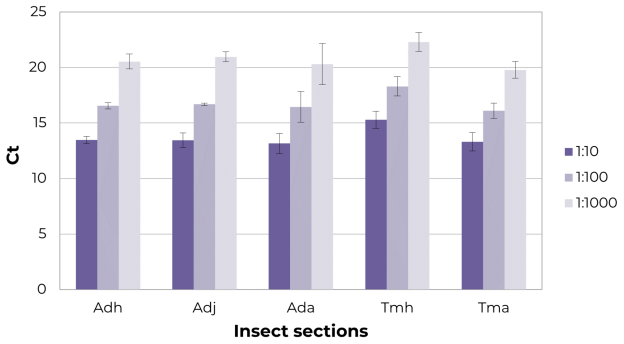

Figure 2. *Ct values obtained from A. domesticus and T. molitor larvae DNA by Real-Time PCR targeting the DNA barcoding regions in the mitochondrial DNA. DNA samples were 1:10, 1:100 and 1:1000 diluted before PCR amplification. The data presented are mean values (n=3, ±1 SD). Adh, A. domesticus head; Adj, A. domesticus jumping legs; Ada, A. domesticus abdomen; Tmh, T. molitor head-thorax; Tma, T. molitor abdomen.*

Discussion

The extraction procedure described here included both chemical/enzymatic and mechanical lysis of the samples by homogenization for highest yields. Depending on the sample input amount and downstream assay, samples can also be lysed by (overnight) incubation at 56°C under shaking. This may lead to slightly lower lysis efficiency but higher integrity of extracted nucleic acids. Extractions were also performed with whole insects, but dilution prior to downstream analysis was necessary to avoid template inhibition in the universal PCR setup used. For more specific targets, it may be beneficial to use whole insects.

A260/A280 ratios of ~1.8 are generally accepted as pure for DNA, while the ratios for pure RNA are ~2. The high A260/A280 purity ratios obtained by NanoDrop dsDNA assay (1.92-2.02) may be affected by co-purified RNA. The combined extraction of DNA and RNA makes it possible to obtain both genomic and transcriptomic information from the same individual insect in one run, allowing for a thorough understanding of the relationship between genotype and phenotype on the basis of diseases and complex traits. However, if it is needed to reduce the amount of DNA or RNA, DNase or RNase A can be added after the lysis incubation.

Conclusion

In summary, we demonstrated that combined extraction of DNA and RNA from insects is feasible with the MagSi-DNA Animal kit. The excellent quality makes the extracted DNA suitable for subsequent PCR or sequencing applications. The extraction protocol can be carried out with minimal equipment requirements, and is easily automated using a magnetic particle processor and/or liquid handling workstation.

Literature

- *Product Manual MagSi-DNA Animal, PM0023, magtivio B.V.*
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