Steinbrenner Laborsysteme GmbH

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MagSi-DNA Animal

Application Note | DNA Extraction from Honey Bees for Parasite Resistance Analysis

Introduction

Honey bees are responsible for up to 80 percent of natural plant pollination worldwide, but also play a key role in pollination for commercial seed production. Mortality of honey bees caused by parasitic varroa mites can cause severe economic damage in commercial beekeeping for honey production or pollination of plants producing seeds. As there is no natural resistance, and the parasite has evolved to become resistant to parasites, attempts are made to breed for resistant or tolerant bee traits to minimize the impact of the varroa mites and to create a stable host parasite relationship.

In addition to phenotypic assays, several genetic markers were identified which are characteristic for the resistant bee traits. Identification of these traits by genotyping assays require purified DNA of good quality which can be used in PCR or microarray assays.

In the current application note we describe the automated purification of DNA from honey bees (*Apis Mellifera*) and the suitability for genotyping assays.

Materials and methods

Honey bees were acquired from a local beekeeper. After calming with a bee smoker, bees were captured and collected into a box, and frozen upon arrival at the lab. Different sections (head, thorax, abdomen) were sampled and added to a 96 U-Bottom DeepWell plate. Lysis was performed using 400 µL buffer TS with 20 µL Proteinase K (20 mg/mL) for 3h at 56°C with shaking at 1000 rpm (Eppendorf, ThermoMixer C).

After incubation, samples were briefly spinned down to collect liquid from condensation and 280 µL lysate was transferred to a 96 DeepWell plate for PurePrep 96. To each sample, 20 µL RNase A (10 mg/mL) was added and samples were incubated for 5 min at RT with shaking at 1000 rpm. DNA purification was performed on the PurePrep 96 System, using the standard kit protocol and reagent volumes as listed in Table 1.

Table 1. *Kit component volumes for DNA purification on the PurePrep 96 System*

Kit component	Volume
Sample lysate	300 µL
MagSi-AG IV	20 µL
Binding Buffer U1	500 µL
Wash Buffer I	2 x 800 µL
Wash Buffer II	800 µL
Elution Buffer	200 µL

After DNA extraction, concentrations 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 was evaluated by automated gelelectrophoresis on the 4150 TapeStation with a Genomic DNA ScreenTape and by Real-Time PCR on the AriaMx Real-Time PCR system (Agilent) with universal primers targeting the beta actin gene. PCR reactions were set up using 4 µL of DNA sample in a total reaction volume of 20 µL (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme).

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Results

DNA concentrations

In Figure 1, DNA concentrations obtained from head, thorax and abdomen sections are presented. The obtained DNA concentrations were between 3.4 ng/µL up to 23.1 ng/µL. Highest concentration were obtained from the abdomen section.

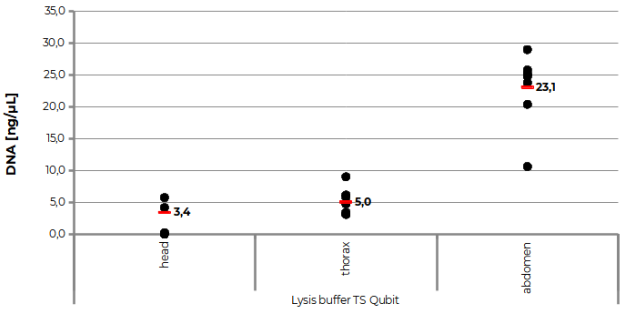

Figure 1. *DNA concentrations (Qubit™ dsDNA BR Assay Kit) obtained after extraction from bee head, thorax or abdomen sections with the MagSi-DNA Animal kit. Data presented are mean values (n=8, ±1 SD).*

Purity

The purity ratios are in the expected ranges for pure DNA (A260/A280 >1.7). Comparable purity ratios were obtained for all samples, but the measurements from the DNA eluates obtained from the head and thorax samples are below the required concentration of 20 ng/µL for reliable A260/A280 and A260/A230 ratios. This results in a higher variability and lower accuracy of the purity measured for DNA from head and thorax sections.

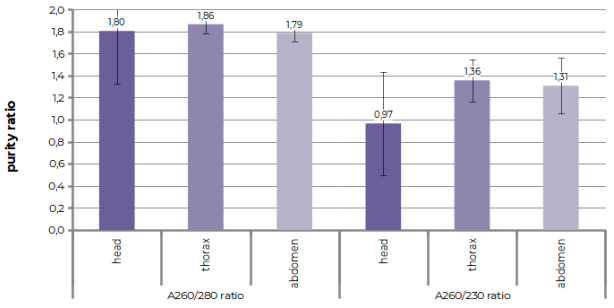

Figure 2. *DNA purity ratios (NanoDrop One) obtained after extraction from bee head, thorax or abdomen samples with the MagSi-DNA Animal kit. Data presented are mean values (n=8, of eight ±1 SD)*

DNA integrity

Results from automated gel electrophoresis are presented in Figure 3. DNA Integrity Numbers ranged from 4.6 to 6.5, indicating only minor DNA degradation related to sample collection and storage. Measured DNA concentrations were 13-25 ng/µL (data not shown) which is in good agreement with the Qubit measurements. All analysed DNA samples show a high amount of high molecular weight DNA with a maximum in size distribution (peak area) of approximately 14,000 bp.

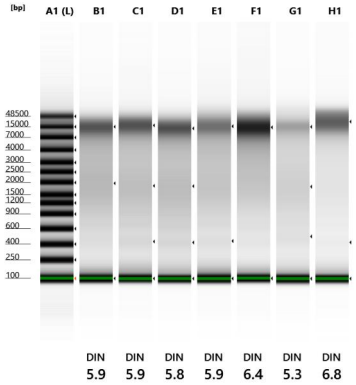

Figure 3. *TapeStation gel image with DNA Integrity Numbers (DIN, 1 - 10) for gDNA extracted from bee abdomen sections. Trace A1: Ladder, Traces B1 to H1: DNA extracted from bee abdomen sections.*

PCR compatibility

Real-Time PCR results are presented in Figure 4 below. Ct values were reported for DNA from all sample types using the without dilution. For the diluted samples the expected increase of the Ct values (approximately 3.3 per 10-fold dilution) was observed on all sample types, indicating the absence of PCR inhibition.

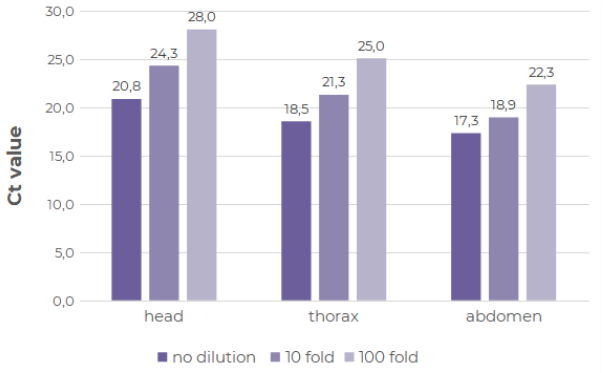

Figure 4. *Ct values obtained for DNA purified from head, thorax and abdomen sections by Real-Time PCR targeting the beta actin-gene. PCR reactions were set up with undiluted, 10-fold, 100-fold and 1000-fold diluted DNA samples. Data presented are mean values (n=2)*

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Conclusion and discussion

The data presented in this application note demonstrates that the MagSi-DNA Animal kit can be successfully used for DNA extraction from bee sections and is suitable for subsequent PCR or microarray-based applications. All DNA concentrations are sufficient, purity values and DNA integrity are good, PCR is successful without dilution and no inhibition is detectable.

The DNA eluates appeared clear and colourless despite of strong colouration of the some sample lysates, indicating high concentrations of unwanted components in the lysate used as input for DNA purification. This demonstrates the high efficiency of the washing procedure of the kit. The extraction procedure included an enzymatic lysis of the sample materials and did not require mechanical sample disruption. In case the maximum yield of DNA is desired, sample homogenization by bead-beating can be used, but it is not recommended due to potential DNA degradation.

The MagSi-DNA Animal kit includes a set of 3 lysis buffers suitable for different sample types. Besides Lysis Buffer TS, for which the data is presented in this application note, Lysis Buffer VT can be used with comparable results. For the current study, the kit performance was exemplified using the PurePrep 96 System, but it is also compatible with other automated magnetic particle processors, e.g. KingFisher™ Flex or liquid handling workstations.

Literature

- *Product Manual MagSi-DNA Animal kit, PM0023, magtivio B.V.*
- *User Guide Qubit™ 1X dsDNA BR Assay, MAN0019617, ThermoFisher Scientific*
- *NanoDrop One UG, 269-309102, ThermoFisher Scientific*
- *DNA Integrity Number (DIN) For the Assessment of Genomic DNA Samples in Real-Time Quantitative PCR (qPCR) Experiments, Application Note 5991-6368EN, A. Padmanaban, Agilent Technologies, Inc.*
- *Toussaint et al., Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments, Journal of Virological Methods 140 (2007) 115–123*

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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