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MagSi-DNA Plant CLS

***Application Note** | DNA extraction from seeds for high-throughput genotyping using the Hamilton MagEx STAR*

Introduction

Plant breeding workflows often use PCR based genotyping, microarrays or Next Generation Sequencing methods to analyse or to identify genetic markers for the optimization of the seed quality. These workflows generate seeds with traits that allow increases in crop productivity, resistance to diseases and drought. Quality inspection of seeds by production companies often includes analysis with PCR-based genotyping using specific genetic marker sets ranging from four to thirty individual markers, depending on the crop species and variety. Seed quality criteria may include a maximum inbred percentage or other criteria requiring large numbers of seeds per batch to be analysed. Although there is variation in yield and quality within and between seed species, it is crucial to obtain DNA of high quality that can be used in such analyses.

MagSi-DNA Plant CLS is intended for fast and cost-effective DNA extractions from all plant samples and includes reagents optimized for processing cotyledon, leafs and seeds. The flexible kit design provides two different lysis buffers (Buffer PL for cotyledon and leaves, Buffer VG for seeds) for an optimized DNA extraction workflow. In addition the procedure is fully scalable to achieve a customized and cost-efficient procedure depending on the requested amount of DNA for down-stream applications.

In the current application note we describe the automated use of the **MagSi-DNA Plant CLS** on the **Hamilton MagEx STAR** workstation. For the processing of the extraction protocol the **Hamilton MagEx STAR** is equipped with four heater shakers and four magnetic separators for Deep-well plates. Up to four plates of 96 samples can be processed in single extraction run without the need for additional external plate storage hotels or refilling of extraction buffers or disposable tips.

In this application note we present exemplary extraction data for the DNA extraction from seeds of six different plant species. DNA quality is analysed using Nanodrop UV spectroscopy, DNA integrity on a Agilent Tape station and qPCR to demonstrate the suitability of the purified DNA for use in genomic applications (e.g. genotyping by PCR or DNA sequencing). Seeds of cucumber (*Cucumis sativus*), tomato (*Lycopersicon lycopersicum*), sweet pepper (*Capsicum annuum*), sugar beet (*Beta vulgaris*), wheat (*Triticum aestivum*), and rape (*Brassica rapa*), were used.

Materials and methods

All seeds used for this study were obtained from a local wholesale market. Seeds from the selected species, were transferred into a 1.4-mL Micronic microtube rack for homogenization. Seeds were mechanically disrupted using 4.7 mm stainless steel beads, in the 2010 Geno/Grinder® (SPEX® SamplePrep), for 5 x 1-min at 1500 rpm) No pretreatment or pre-incubation of the seeds in lysis buffer or water were applied before homogenization.

After homogenization, 600 µL Lysis Buffer VG or Lysis Buffer PL was added (both supplemented with RNase A), samples were briefly mechanically resuspended (1 min at 1500 rpm (GenoGrinder®) and finally incubated at 65 °C for 60 min in a Binder E28 drying oven. Disrupted and lysed samples were centrifuged for 15 min (6000 x g) to pellet cell debris. Afterwards 400 µL sample lysate was transferred and used as input for automated DNA purification on the Hamilton MagEx STAR instrument with a final elution volume of 150 µL.

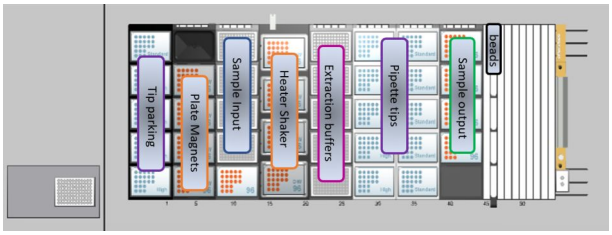

***Figure 1.** Deck layout of the Hamilton MagEx STAR instrument (Figure taken from Hamilton Venus software)*

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DNA concentrations and purity of the eluates were measured by UV-VIS with the NanoDrop™ One according to manufacturer's instructions (Thermo Fisher Scientific™). The presence or absence of inhibitors was evaluated by qPCR on the AriaMx Real-Time PCR system (Agilent Technologie) with primers targeting the tRNA-leucine gene. From the samples, 2 µL undiluted and 1:10 and 1:100 diluted DNA were used in a total reaction volume of 20 µL (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme). DNA integrity was analysed using the 4150 Tape-Station (Agilent technologies) using the Genomic DNA (gDNA) ScreenTape assay using 1 µL of undiluted extracted DNA per sample.

Results

Throughput / Run time

With the current Hamilton method up to four extraction plates of 96 samples can be processed within 90 min. The purification process for 384 samples requires 384 standard volume tips and 392 high volume tips.

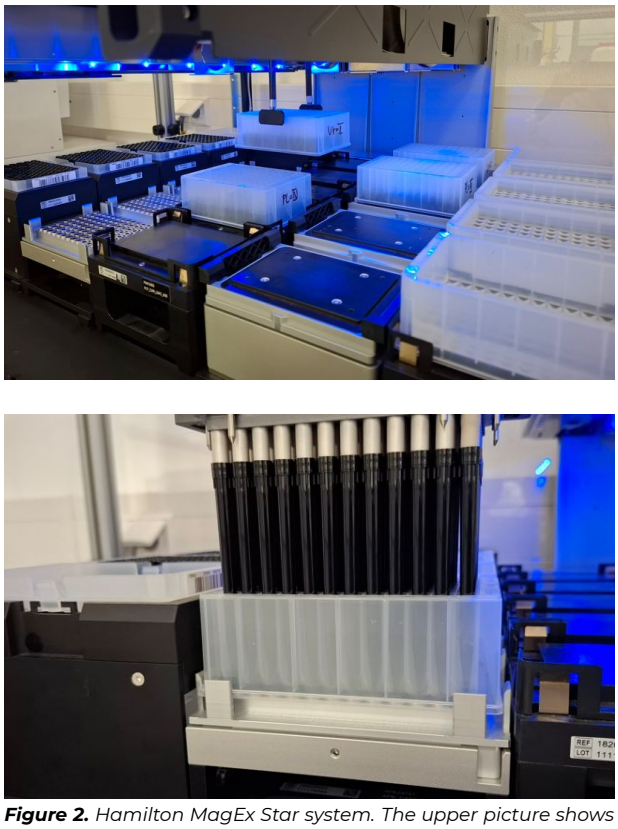

Figure 2. *Hamilton MagEx Star system. The upper picture shows a plate transportation step to the Hamilton Heater Shaker module using the CO-RE Gripper tool. The lower picture shows a liquid aspiration step form one of the four processing plates placed on a MM-Separator 96 DeepWell magnet using the CO-RE 96 Multi-Probe Head equipped with 1 mL disposable tips.*

DNA concentration and purity

Average DNA concentrations from exemplarily selected extracts of all seeds tested are presented in Figure 3. DNA concentrations were highly dependent on the plant species and seed size, and ranged from 6 to 30 ng/µL in a recovered elution volume of 150 µL. Typically the Lysis Buffer VG is recommended for seeds. However, the Lysis Buffer PL which is usually recommended for leaves may be considered to be used for seeds as well. Depending on the plant species Lysis Buffer PL generates higher concentrations (pepper, tomato and cucumber) in comparison to Lysis Buffer VG. All measured A260/A280 DNA purity ratios were ≥1.6, indicating high quality DNA data not shown).

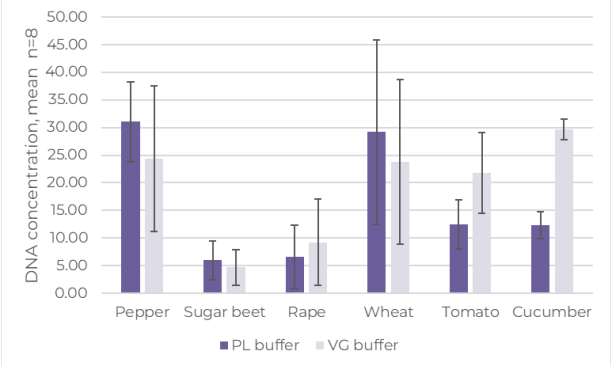

Figure 3. *DNA concentrations obtained from various seeds, measured by UV-VIS with the NanoDrop™ One. The data are presented as mean (n=8, ±1 SD).*

DNA integrity

In Figure 4, DNA integrities determined by Agilent Genomic ScreenTape Assay of the extracted DNA samples are presented. Extracts from multiple samples were pooled together and analysed. A considerable amount of high molecular weight DNA (>14 kbp to up to >60 kbp) is observed for most of the tested plant species.

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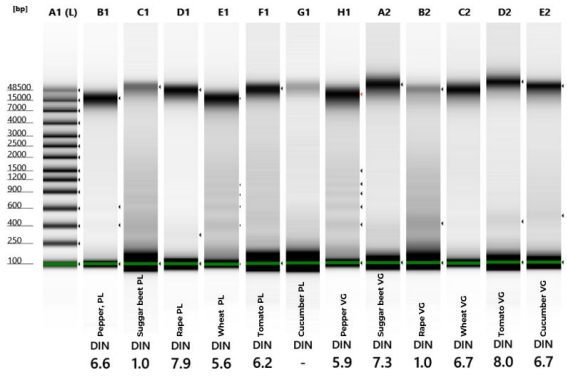

Figure 4. *Integrities of the purified DNA (Genomic Screen Tape assay. Exemplarily samples were pooled (n=8) and subsequently analysed.*

qPCR

qPCR results are presented in Figure 5. Ct values were reported for all samples without dilution, 10 fold dilution and 100 fold dilution. Dilution of DNA samples resulted in the expected increase of the Ct value (ΔCt ~ 3.3 per 10-fold dilution), indicating the absence of PCR inhibition.

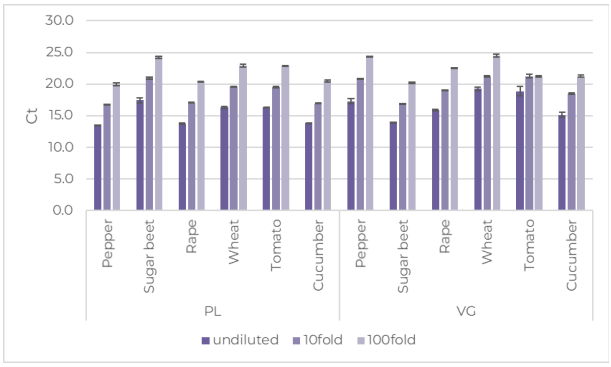

Figure 5. *qPCR results from extracted vegetable, field crop and oil seed DNA with primers targeting the tRNA-leucine gene. The data are presented as mean (n=2, ±1 SD).*

Discussion and conclusion

The data presented demonstrates that DNA can be successfully extracted from a variety of seeds using the MagSi-DNA Plant CLS kit on a Hamilton MagEx STAR workstation. The variations in obtained DNA concentrations, within and between seed types, are related to natural differences such as size and weight. However, high quality DNA was obtained from all seeds which is suitable for analysis by Real-Time PCR. The final volume of purified sample DNA allows for a high number of individual assays to be performed. When requiring higher DNA concentrations, e.g. for genotyping by sequencing (GBS), elution volumes can be adjusted in the automated DNA extraction protocol.

It can be concluded that MagSi-DNA Plant CLS provides a suitable extraction method for DNA extraction from seeds for genotyping assays by seed production companies and other agriculture laboratories. The scalable and flexible extraction protocol can be easily automated on a Hamilton MagEx STAR workstation with the standard equipment. Especially the use of the CO-RE 96 Multi-Probe Head with the ability to use 1 mL pipette tips speed up the extraction procedure.

Literature

- *Product Manual MagSi-DNA Plant CLS, magtivio B.V.*
- *NanoDrop One UG, 269-309102, ThermoFisher Scientific*
- *Dwivedi et al, 2020. First the seed: Genomic advances in seed science for improved crop productivity and food security. Crop Science. 2021; 61:1501-1526.*
- *Verma et al, 2022. Application of Recombinant DNA technology in Agriculture: A Review. International Journal of Advanced Research in Biological Sciences. 9(3): 138-146.*
- *Lauba et al., 2010. Development of primer and probe sets for the detection of plant species in honey. Food chemistry. 2010; 118: 979-986.*

Ordering information

Art. No.	Description	Amount
MDKT00260096	MagSi-DNA Plant CLS	96 preps
MDKT00260960	MagSi-DNA Plant CLS	10 x 96 preps
Bulk deliveries are available on request		

For inquiries and orders or technical support please contact sales@magtivio.com.

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