Efficient DNA extraction from food and feed

Rapid extraction of genomic DNA from a variety of food and feed samples with NucleoSpin® 8/96 Food columns and the Freedom EVO®

Introduction

In the past decade, the rise of molecular diagnostics in research and quality control has greatly increased the need for reliable and reproducible extraction of nucleic acids from food and feed. Food samples are very heterogeneous, and often contain substances which can impair DNA extraction, such as fats, cocoa or polysaccharides. In addition, processed foods often have a very low DNA content, which is of poor quality. It is therefore important that PCR inhibitors are completely removed, to enable even low amounts of partially degraded DNA to be extracted from these complex samples.

MACHEREY-NAGEL has developed the NucleoSpin Food kit to fulfill the need for fast extraction of small genomic DNA fragments from a variety of food samples of plant or animal origin. The filter-based system maximizes the yield of high quality DNA, while maintaining workflow flexibility with regard to scalability (amount of starting material) and sample numbers.

Tecan and MACHERY-NAGEL have joined forces to provide an efficient automated solution for the isolation of genomic DNA from foods, without compromising yield or purity. Full automation on the Freedom EVO workstation provides rapid, reliable extraction of high quality genomic DNA from food and feed samples, delivering high yields of equal or superior quality compared to manual processing and accommodating either the native food sample or sample lysate, depending on the type of food sample to be analyzed. The risk of contamination, carry-over and manual errors is minimized, and the overall process security is further enhanced by sample tracking.

Materials and Methods

Equipment

An overview of the equipment required is given in Table 1. The Freedom EVO workstation can be equipped with a 2-, 4- or 8-channel Liquid Handling (LiHa) Arm using disposable tips adaptors and a low tip ejection option to reduce the risk of cross-contamination. A Robotic Manipulator (RoMa) Arm, a Te-Shake™ module (for optimal mixing of samples and buffers) and a Te-VacS™ module (which can process both 96-well plates and 8-well strips, Figure 1) enable full process automation.

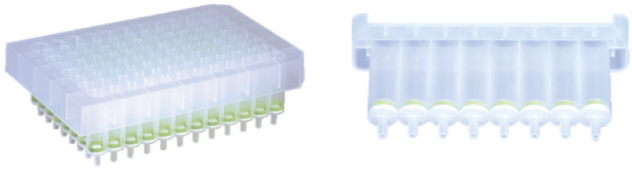

Figure 1 MACHEREY-NAGEL 96-well plate and 8-well strip.

Sample numbers	8 to 96 samples per batch (in increments of 8)
Batch time	50 mins for 48 samples (8-well strips) 1 h 04 mins for 96 samples (96-well plate)
Equipment Tecan	• Freedom EVO 100 platform, 8-channel Liquid Handling Arm configured for disposable tips, 1,000 µl syringes, Robotic Manipulator Arm, Te-VacS, Te-Shake, stainless steel deck and safety panel set • Tube, trough and disposable tip carriers • Wash station with waste disposal • Disposable tips (filtered) 1,000 µl • 200 µl and 100 ml troughs • Freedom EVOware® Standard software
Equipment MACHEREY-NAGEL	• NucleoSpin 8 / 96 Food kit • Square-well plates • Starter Set A (required for 8-well strips only)

Table 1 Overview of equipment required for DNA extraction from food samples.

Automated workflow

Food samples (up to 200 mg) are homogenized using the appropriate protocol for each food type. Lysis can be performed manually or integrated into the automated procedure, depending on the individual laboratory workflow, and the lysates introduced onto the Freedom EVO workstation in either a 96-well plate or 1.5 ml tubes. The fully automated DNA extraction procedure includes DNA binding to NucleoSpin Food columns, washing, and the final elution of the purified DNA in volumes of 100 – 200 µl; the experiments described in this application note use 2 x 80 µl elution volumes.

The configuration and scripting of the Freedom EVO workstation have been optimized to minimize the risk of cross-contamination and maximize the yield of nucleic acids.

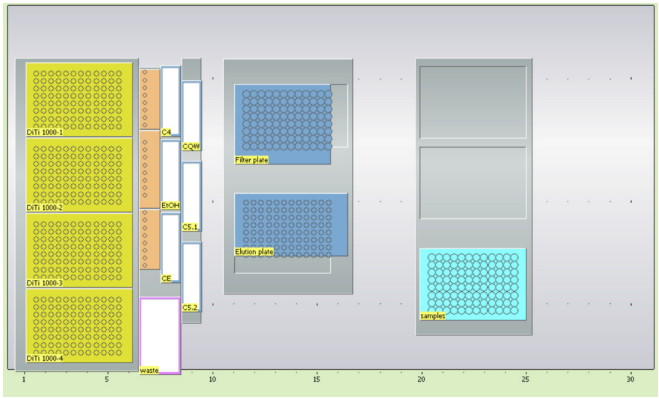

Figure 2 Worktable layout for high throughput isolation of DNA from food and feed using the Te-VacS module.

Results

Automation of the NucleoSpin Food kit on the Freedom EVO workstation allows for fast, convenient and reliable purification of genomic DNA from foodstuffs.

Processing time

The extraction of genomic DNA from food and feed samples, starting with the lysed material, is very rapid. On average, genomic DNA can be extracted from 96 samples from foods such as wheatmeal – either manually or by an automated procedure (96-well format) – in about 1 h. If 8-well strips are used, 48 samples can be processed in just 50 mins.

Yield and reproducibility

Automated DNA extraction on the Freedom EVO workstation is highly comparable with manual processing in respect of yield and purity. The typical yield of 6 – 7 µg per 200 mg of wheatmeal material using the automated process is equal to or higher than with the manual process (Figure 3).

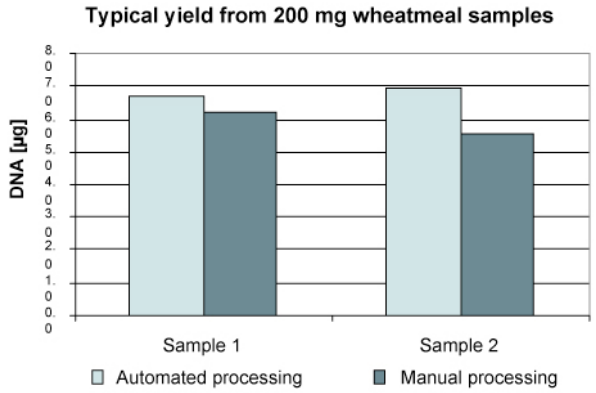

Figure 3 Comparison of DNA yield using the automated workflow on the Freedom EVO and the manual process. DNA was isolated from 200 mg aliquots of two independent wheatmeal samples.

DNA extraction on the Freedom EVO workstation gives a highly reproducible yield and a low CV of 7.12 % (Figure 4).

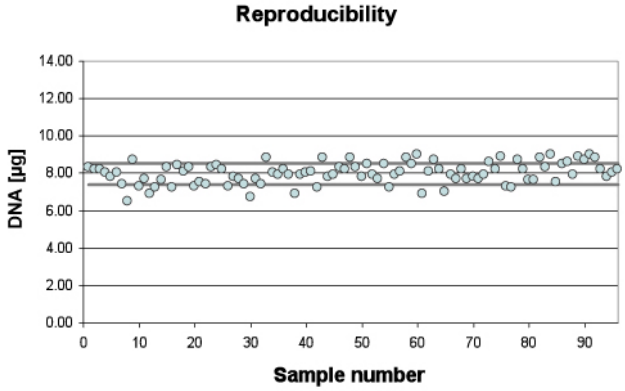

Figure 4 The automated workflow on the Freedom EVO results in a highly reproducible DNA yield with a CV of 7.12 %.

In general, the DNA yield strongly depends on the type of food material, as different food samples contain varying amounts of DNA (Figure 5).

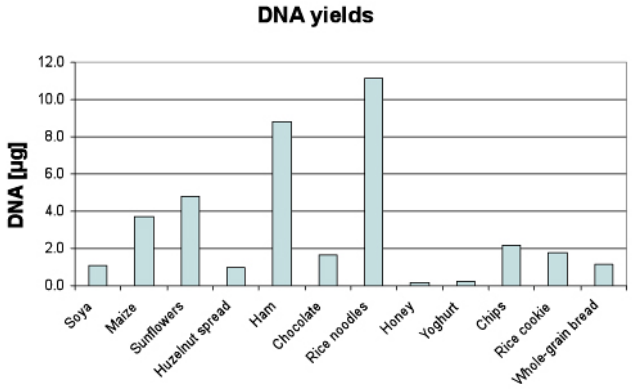

Figure 5 Comparison of the DNA yields from 200 mg samples of a variety of foods.

Purity

With A_{260}/A_{280} ratios averaging 1.89 for wheatmeal (Figure 6), the eluted DNA is of excellent purity, and is therefore suitable for all common downstream applications.

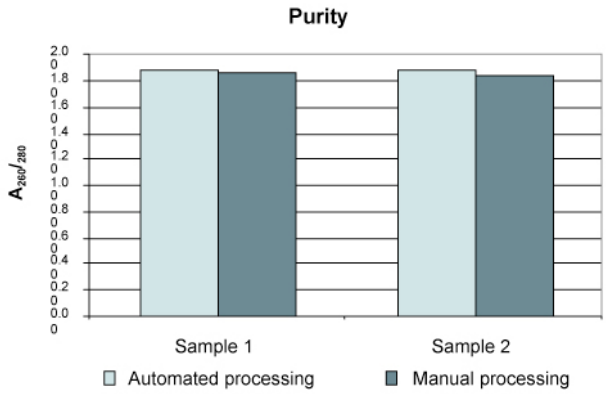

Figure 6 Purity of DNA extracted from 200 mg wheatmeal samples using automated and manual processing.

Downstream applications and cross-contamination

DNA from a variety of sample sources – ranging from corn, sunflowers and bread, to rice noodles and rice cookies, ham and wholegrain bread – was extracted, and 2 µl of each DNA eluate was amplified in a 40 cycle, real-time PCR for tRNA leucin (Roche LightCycler®, SYBR® Green assay, Figure 7). The resulting 74 bp fragments of tRNA leucin were subjected to gel electrophoresis and subsequent staining, as shown in Figure 8.

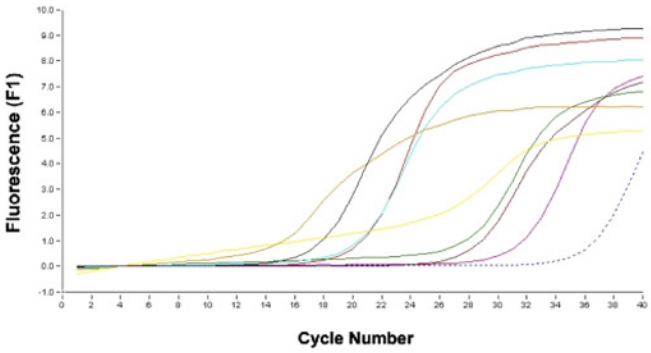

Figure 7 DNA from corn, soya, chips, sunflowers, rice noodles, ham, rice cookies and wholegrain bread was extracted and subjected to real-time PCR for tRNA leucin (Roche LightCycler). The dotted line represents the negative control (product formation due to unspecific primer dimer amplification).

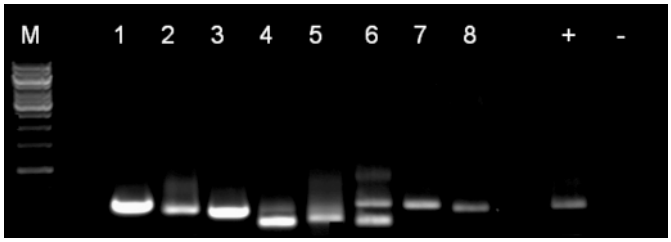

Figure 8 tRNA leucin from a variety of food samples was amplified and subjected to gel electrophoresis on a 1 % TAE Gel; M = Lamda HindIII Marker (Fermentas), 1: soya, 2: corn, 3: sunflowers, 4: ham, 5: rice noodles, 6: chips, 7: rice cookies, 8: wholegrain bread.

To exclude cross-contamination, DNA was extracted using the NucleoSpin Food protocol and a square-well plate filled with a checkerboard pattern of wheatmeal samples and PBS buffer (negative control). No amplification was detected in the negative samples, indicating the absence of cross-contamination.

Conclusion

Automation of the NucleoSpin Food kit on a Freedom EVO workstation allows rapid, reproducible extraction of genomic DNA from a variety of food and feed samples, offering food laboratories a reliable, truly walkaway solution.

The Freedom EVO platform can be equipped with a range of optional modules, such as absorbance readers, storage modules and cooling devices, providing maximum flexibility to meet the ever-changing needs of the laboratory.

Talk to your local Tecan representative to customize the Freedom EVO workstation to your laboratory's needs.

Acknowledgements

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Further Application Notes

Updated list at www.tecan.com/machereynagel

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