MACHEREY-NAGEL

NucleoSpin® Food

Reliable purification of DNA from a variety of commercially important crop seeds

Introduction

Whether as a tool for developing of new breeds through marker-assisted selection and hybridization or as a way of tracking the presence of GMO in crops, genotyping is playing an increasingly important role in modern food production. This is especially relevant in cultivating seeds and grains.

Direct genotyping of seeds saves time and resources required for plant germination and allows for analysis prior to planting season. Extracting sufficient amounts of high quality DNA from a variety of seed samples is therefore an important prerequisite for commercial genotyping of crops.

However, extracting DNA from seeds is complicated by the presence of nutritional macromolecules as well as the inherently low amounts of DNA in the sample. Methods for extracting DNA from seed samples tend to be either laborious and complicated or deliver low quality DNA unsuited for many PCR applications.

MACHEREY-NAGEL has developed NucleoSpin® Food for DNA extraction from diverse, challenging food matrices. Due to its robust buffer chemistry and a resilient silica membrane, the kit has proven to work with a variety of challenging samples. This application note demonstrates that NucleoSpin® Food can be used for routine extraction of high quality DNA suitable for PCR analysis from a variety of commercially important seed samples. Furthermore, a single protocol can be applied to all seed samples, allowing for even simpler processing.

Materials and methods

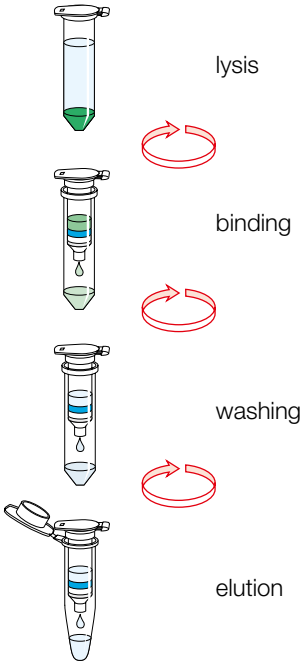
For each sample, 200 mg of material was homogenized in liquid nitrogen with mortar and pestle. Sample lysis and subsequent DNA isolation were performed following the standard protocol of NucleoSpin® Food. Elution was performed with 100 µL of elution buffer CE. For each eluate, 10 µL were used for spectrophotometric analysis and 2 µL were analyzed on a 1 % TAE agarose gel. A further 1 µL was diluted 1:100 and analyzed by qPCR (1 µL of the diluted sample in a 20 µL reaction mix) with specific primers.

Product at a glance

General properties of NucleoSpin® Food

NucleoSpin® Food	
Technology	Silica membrane technology
Format	Mini spin columns
Sample material	5–200 mg
Fragment size	300 bp–approx. 50 kbp
Typical yield	0.1–10 µg (200 mg food)
A_{260}/A_{280}	1.6–1.9
Elution volume	100 µL
Preparation time	30 min/6 preps
Binding capacity	30 µg

NucleoSpin® Food procedure

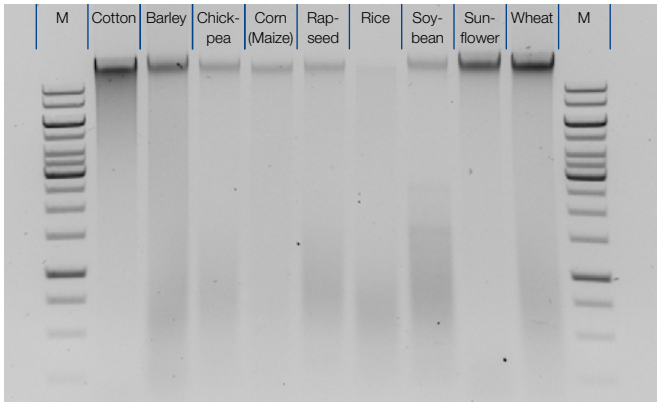


Application data

High DNA yields from various seed samples

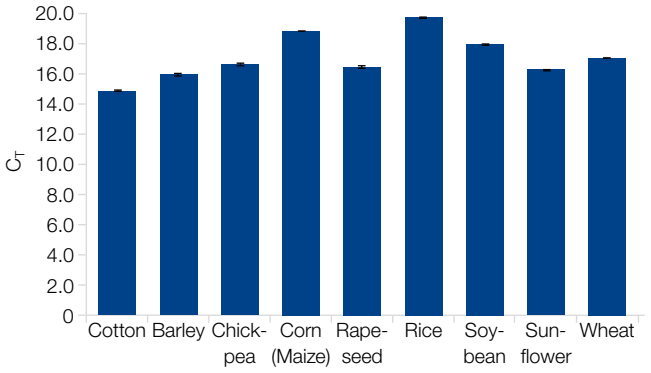
Several seed samples were prepared according to the standard protocol of NucleoSpin® Food. For each sample, 10 µL of eluate were analyzed by spectrophotometer. Successful isolation of DNA could be demonstrated in each case.

Sample	Species name	Yield (µg)
Cotton	*Gossypium herbaceum*	91
Barley	*Hordeum vulgare*	106
Chickpea	*Cicer arietinum*	61
Corn (Maize)	*Zea mays*	154
Rapeseed	*Brassica napus*	92
Rice	*Oryza sativa*	79
Soybean	*Glycine max*	113
Sunflower	*Helianthus annuus*	60
Wheat	*Triticum aestivum*	66



Reliable extraction of DNA from various seed matrices

After spectrophotometric analysis, DNA purified from several seed samples was further examined by agarose gel electrophoresis. For each sample 2 µL of eluate were loaded onto a 1 % agarose gel. The marker (M) was 1 kB ladder (Fermentas). While the amounts of extracted DNA varied between different sample types, in each case detectable amounts of DNA could be reliably extracted from 200 mg of sample.



Amounts and quality of the extracted DNA were sufficient for detection by qPCR

The extracted samples were analyzed by qPCR with the Eu +/- marker. The purity and concentration of DNA were sufficient for reliable detection of the genetic marker and thus suitable for genotyping.

Summary

NucleoSpin® Food allows routine extraction of high quality DNA from even the most difficult seed samples. The high yields and purity of the extracted DNA lead to accurate and reliable results from downstream analyses such as PCR, including qPCR. This in turn permits quick and affordable genotyping of highly important crops such as rice, maize, wheat and soybean.

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® Food	NucleoSpin® Food Columns, Collection Tubes (2 mL), buffers, Proteinase K	10 / 50 / 250	740945.10 / .50 / .250