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Automated Purification of Genomic DNA from Plants with the Macherey-Nagel NucleoMag® Plant Kit on the ep*Motion*® 5075

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Abstract

The purification of genomic DNA from plant samples is routinely performed in a variety of research laboratories of food and agriculture with a growing demand for automation. Automation significantly facilitates the purification, especially for versatile sample material like leaves, seeds and a lot of other materials.

The NucleoMag 96 Plant Kit from Macherey-Nagel is adapted to the ep*Motion* 5075t/m. The combination of epMotion 5075t/m and the purification kit, allows a walk away purification of DNA from 96 plant samples in less than 100 minutes.

Introduction

DNA extraction from plant material becomes increasingly important for food analysis, crop improvement and plant health research. Different parts of plants, such as seeds, leaves or even parasitic fungi, can be used for the purification of genomic DNA. The procedure of the NucleoMag 96 Plant kit is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Plant tissue is homogenized and subsequently lysed with CTAB-based lysis buffer MC-1. The contaminants are removed through several washing steps with wash buffers MC3 and MC4. Salts are then removed with additional wash step with 80% ethanol, which replaces the MC5 wash buffer. The purified DNA is then eluted and can be used directly as a template for qPCR, next generation sequencing, or any kind of enzymatic reactions.
This application note describes the configuration and preparation of the ep*Motion* 5075 to automate this kit.

Materials and Methods

Required Labware
Eppendorf ep*Motion* 5075t or 5075m
Dispensing Tool TM 1000-8
Dispensing Tool TM 300-8
Reservoir Rack
Reservoirs 30 mL/ Reservoirs 100 mL
Reservoir 400 mL
NucleoMag Sep (Magnetic separator)
NucleoMag 96 Plant Kit

Required Consumables
epT.I.P.S® Motion 1000 µL with filter
epT.I.P.S Motion 300 µL with filter

Samples
Wheat leaves and seeds
Maize leaves and seeds

Method
This protocol is programmed to process up to 96 samples in parallel. This method was developed for the ep*Motion* 5075m or 5075t.

This kit is suitable to process up 50 mg wet weight of plant tissue. The plant samples can be fresh or from storage – either frozen, under ethanol or lyophilized. When using dried material, the sample amount needs to be reduced to 10 mg, but may simplify the extraction process and yield more DNA. The lysis procedure is most effective with well homogenized, powdered samples. Different methods can be used for sample preparation, like grinding with pestle in the presence of liquid nitrogen or other commercial homogenizers e.g. bead mill.This needs to be adjusted to the origin and type of sample material. Prior to automated procedure the following steps have to be carried out manually. In each sample vessel 20 to 50 mg of homogenized plant material, 500 µL lysis Buffer MC 1 and 10 µL RNase A are incubated in a shaker at 56°C for 30 minutes. After clearing the lysate in a centrifugation step, 400 µL of the clear lysate is transferred into each well of a separation plate. All subsequent steps are automated in this plate. This includes dispensing of buffers and beads, removal of the supernatant as well as all transport and mixing steps. After the last washing step residual ethanol is removed in a drying step of 10 minutes at 56°C. Finally, the eluate will be transferred to a dedicated elution plate.

For the method the following positions of the worktable are occupied:

Position	Labware	Comment
A2	300 µL filtertips	
A3	300 µL filtertips	
TMX	Separation Plate (Lysed samples)	
B1	1000 µL filtertips	
B2	1000 µL filtertips	
B3	1000 µL filtertips	
C2	Liquid Waste (400 mL reservoir)	
C3	NucleoMag_Sep	
C4	Reagent reservoirs	
C5	Elution Plate	

Figure 1: ReservoirRack Layout

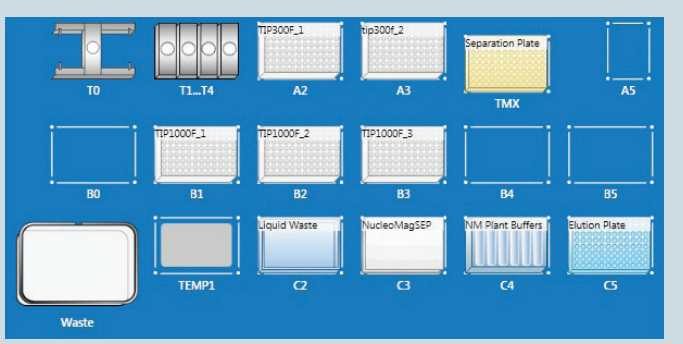


Figure 2: Worktable allocation

Results and Discussion

Purification results from wheat and maize leaves:
Genomic DNA acquired with the aforementioned method was analyzed by gel electrophoresis of 10 µL eluate or PCR product via 1% TAE agarose gel; yield and purity were determined by UV spectroscopy- with Synergy™ HT Multi-detection microplate reader (BioTek®). Furthermore a qPCR with SensiFast® Probe Lo-Rox Kit (Bioline®) on an Applied Biosystems® 7500 instrument was used to check for the absence of inhibitors.

Wheat leaves and maize leaves

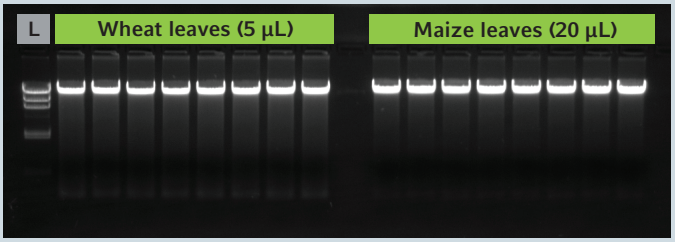
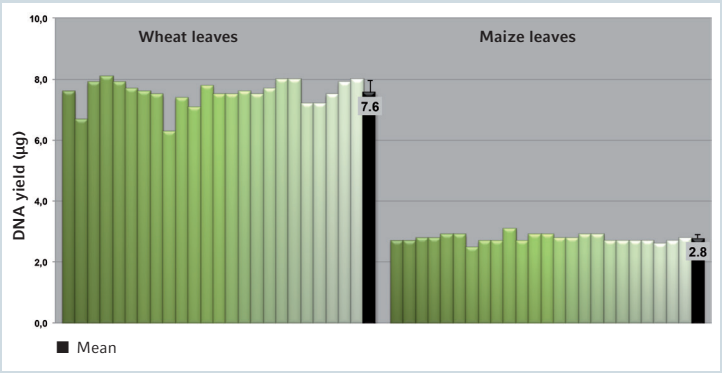


Figure 3: 5 or 20µL of the final eluate from wheat or maize leaves were subjected to agarose gel electrophopresis on a 1 % gel. Running time was 30 minutes at 100 V.



	Wheat leaves			Maize leaves		
DNA yield (µg)	7.6	6.3	7.7	2.7	2.7	2.8
	6.7	7.4	8.0	2.8	2.7	2.7
	7.9	7.1	8.0	2.9	2.9	2.7
	8.1	7.8	7.2	2.9	2.9	2.7
	7.9	7.5	7.2	2.5	2.8	2.7
	7.7	7.5	7.5	2.7	2.8	2.6
	7.6	7.6	7.9	2.7	2.9	2.7
	7.5	7.5	8.0	3.1	2.9	2.8
MEAN		**7.6**			**2.8**	
STDEV		**0.4**			**0.1**	

Figures 4 and 5: DNA yields determine with Synergy HT Multi-detection microplate reader, BioTek

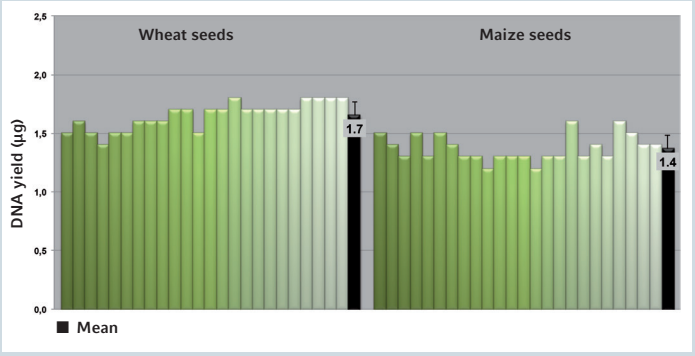
	Wheat leaves			Maize leaves		
DNA Purity (260/280 nm)	1.8	1.8	1.9	1.4	1.5	1.4
	1.8	1.9	1.9	1.3	1.4	1.4
	1.8	1.9	1.9	1.4	1.4	1.4
	1.8	1.8	1.8	1.4	1.4	1.3
	1.9	1.7	1.9	1.4	1.4	1.4
	1.9	1.8	1.9	1.4	1.4	1.4
	1.9	1.9	1.9	1.4	1.4	1.4
	1.8	1.9	1.9	1.4	1.4	1.4
MEAN		1.8			1.4	
STDEV		0.1			0.1	

Figures 6 and 7: DNA purity determine with Synergy HT Multi-detection microplate reader, BioTek

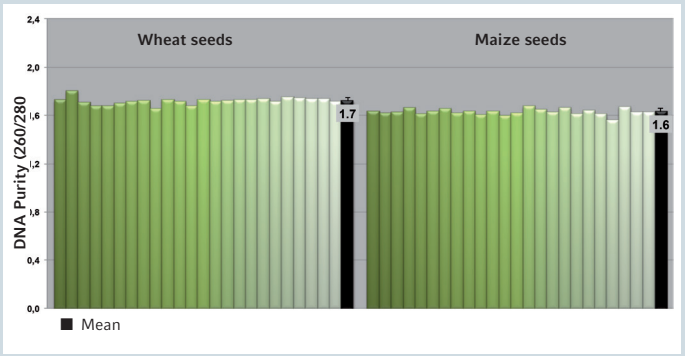
Wheat and Maize seeds

Purification results from seeds: Yield and purity of the genomic DNA acquired with the aforementioned method were determined by UV spectroscopy with Synergy HT Multi-detection microplate reader (BioTek).

Furthermore a qPCR with SensiFast Probe Lo-Rox Kit (Bioline) on an Applied Biosystems 7500 was used to check for the absence of inhibitors.



Figures 8: DNA yield determined with Synergy HT Multi-detection microplate reader, BioTek



Figures 9: DNA purity determined with Synergy HT Multi-detection microplate reader, BioTek

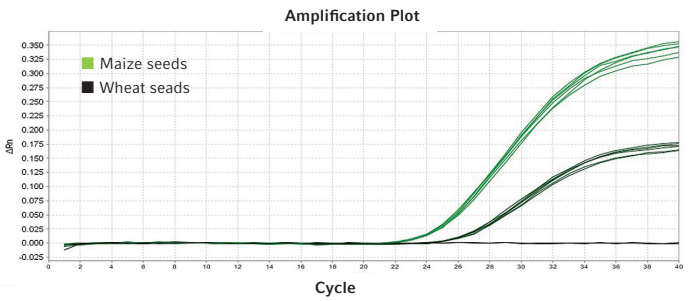


Figure 10: 4 µL selected eluates were assayed in a quantitative PCR with a Taqman® probe for 100 bp amplicon of beta-Actin, using the SensiFast Probe LoRox Kit (Bioline) on an ABI 7500 device.

Crosscontamination

The purified genomic DNA is suitable for a full range of downstream methods. The results from the electrophoresis analysis, qPCR, purity and yield as well as the proven absence of cross-contamination show the performance of the described automation of the NucleoMag 96 Plant Kit on the ep*Motion* 5075t/m. Similar results can be achieved with a great variety of plant species and tissue types.

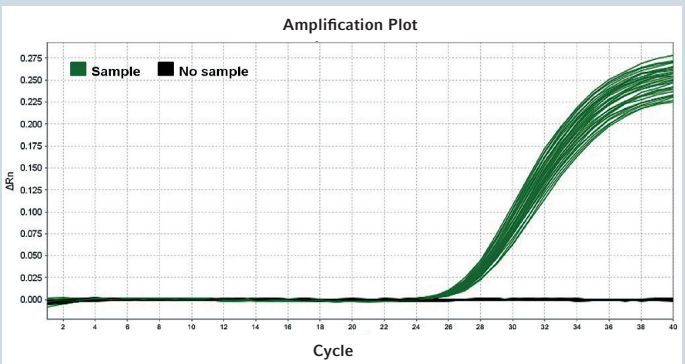


Figure 11: 4 µL of the eluates from wells containing lysate as well as eluates from wells without lysate were assayed in a quantitative PCR with a Taqman® probe for a 100 bp amplicon of beta-Actin, using the SensiFast Probe LoRox Kit (Bioline) on an Applied biosystems 7500 device. No amplification was observed in the negative extraction controls.

Ordering information

Description	Order no. international
ep*Motion*® 5075t	5075 000.302
ep*Motion*® 5075m	5075 000.305
ReservoirRack	5075 754.002
TM 1000-8 Dispensing tool	5280 000.258
TM 300-8 Dispensing tool	5280 000.231
epT.I.P.S® Motion 1000 µL SafeRack with filter	0030 014.650
epT.I.P.S® Motion 300 µL with filter	0030 014.456
Reservoir 30 mL	0030 126.505
Reservoir 100 mL	0030 126.513
Reservoir 400 mL	5075 751.364

Macherey-Nagel	
NucleoMag® 96 Tissue	REF 744400
NucleoMag® SEP	REF 744900
Square well block	REF 740481

Your local distributor: www.eppendorf.com/contact
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