Applications

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Automated Plant DNA Purification using the Nucleon® Plant DNA Kit on the ep*Motion*® 5075 TMX from Eppendorf

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Abstract

In the field of plant molecular biology successful analysis requires the purification of reliable and high quality genomic DNA. The purification of such DNA can be time consuming particularly in large scale projects where many individual samples need to be processed. A reliable, reproducible and easy to handle automated method for the purification of plant derived DNA is therefore very desirable. In this application note we demonstrate an automated method for the purification of plant genomic DNA using the Nucleon Plant DNA Kit from Gen-Probe Life Sciences Ltd. and the Eppendorf liquid handling workstation ep*Motion* 5075 TMX. A range of plant materials were subjected to the automated purification procedure and analysed for yield, quality and cross sample contamination. The automated method was found to deliver consistently high purity, high molecular weight DNA from the sample types tested with a mean 260/280 absorbance ratio of 2.05. The average total yield derived from a small amount of starting plant material (30 mg) was found to be 11.65 µg. With a protocol time of 4 hours (with minimal hands on time) for 96 samples and no detectable cross sample contamination the methodology provides a viable easy to use automated procedure for the purification of plant derived DNA.

Introduction

The Nucleon Plant DNA Kit is designed for the rapid, economical purification of high quality, high yield DNA from a wide range of plant materials. The purification process is based on the reversible binding of DNA to novel magnetic beads. The beads, which are not silica coated, have nanometre dimensions, which can aggregate to form functional complexes. These complexes provide the means to purify DNA from complex mixtures and provide consistent quality and yield.

The ep*Motion* 5075 TMX is the unit with the most flexible range of applications in the ep*Motion* family of automated pipetting systems. It includes an integrated TMX module to shake and heat samples which can be loaded and unloaded with the gripper.

Pipetting to the mixer is possible before and after mixing steps. In combination with the Eppendorf ep*Motion* TMX workstation the Nucleon Plant DNA kit can be easily automated. The integrated TMX module means that following sample disruption the lytic process, that is enhanced by incubation at higher temperatures, can be performed online and further reduces the need for hands on time. This incubation step is instrumental in delivering higher yield and purity DNA. Following lysis the magnetic beads are mixed with the samples and lysate transferred to the purification block for the commencement of the automated purification process. The following steps of DNA binding, washing, drying and elution are fully supported by the ep*Motion* TMX system delivering the final ready to use eluted high quality, high yield DNA for enhanced performance in downstream applications such as restriction digestion.

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eppendorf

Materials and Methods

ep*Motion* 5075 TMX with gripper and TMX Module [1]
Dispensing tool TM 1000-8
Multi Magnetic Probe Plate
Promega Magnabot 96 magnetic Separation Device
A 'bead beater' or 'mixer mill'
Centrifuge
Vortex
Nucleon Plant DNA Kit [2]

Sample Material

Cabbage: Leaf (cut)
Pine: Leaf (Cut)
Rose: Leaf (cut)
Lettuce: Leaf (cut)
Corn: Popping corn (powdered)
Barley: Leaf (cut)

Reagent Preparation

Plant Wash Buffer

105 mL of 96 % ethanol was added to the Plant Wash Buffer (B) bottle.

Magnetic Particle / RNase A Suspension

28.5 mL of 96 % isopropanol was added to the container for the preparation of the Magnetic Particle Suspension. The Magnetic Particles in the 2 mL vial were completely resuspended by vortexing thoroughly before transferring all the contents into the bottle for the Magnetic Particle Suspension. The container was sealed with the lid and shaken to ensure complete resuspension of the Magnetic Particles. 9.9 mL of 96 % isopropanol was added to the container for the preparation of the RNase A Suspension. This was added to the magnetic particle suspension container and mixed.

Lysis Solution

The contents of a vial of proteinase K were added to a bottle of plant lysis solution (A). The bottle was swirled gently to ensure the powder fully dissolved.

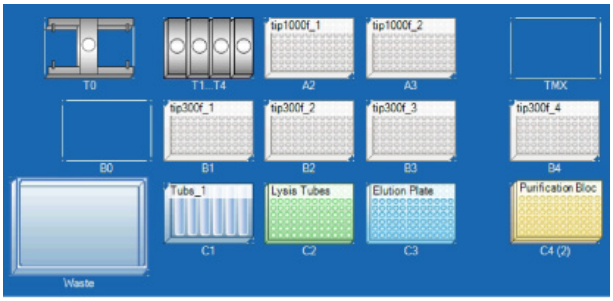

Fig. 1: Screen shot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 TMX worktable for use with the Nucleon Plant DNA Kit.

Automated sample processing

The ep*Motion* 5075 TMX worktable was prepared according to Figure 1. The detailed information on the required labware for each position on the worktable can be found in Table 1.

Table 1: ep*Motion* 5075 TMX worktable details for the Nucleon Plant DNA Kit protocol.

Position	Labware	Comment
T0	Gripper	
T1-T4	1000 µL Multi Dispenser, 300 µL MultiDispenser	
A2	epTIPS Motion 1000 µL Filter Eppendorf	96 tips for 96 samples
A3	epTIPS Motion 1000 µL Filter Eppendorf	96 tips for 96 samples
TMX	Empty	
B0	Empty	
B1	epTIPS Motion 300 µL Filter Eppendorf	96 tips for 96 samples
B2	epTIPS Motion 300 µL Filter Eppendorf	96 tips for 96 samples
B3	epTIPS Motion 300 µL Filter Eppendorf	96 tips for 96 samples
B4	epTIPS Motion 300 µL Filter Eppendorf	96 tips for 96 samples
WASTE	Waste	
C1	Position 1: Lysis Buffer (A) Position 2: Neutralisation Buffer (C) Position 3: Magnetic Beads & RNase A Solution Position 4: Wash Buffer (B) Position 5: Elution Buffer (D) Position 6: Empty (for waste) Position 7: Empty (for waste)	30 mL Reservoir 30 mL Reservoir 100 mL Reservoir 100 mL Reservoir 30 mL Reservoir 100 mL Reservoir 100 mL Reservoir
C2	Cluster Tubes containing plant material	
C3	Microplate 96/U Eppendorf	For elution
C4	Magnabot Magnetic Separation Stand Promega (bottom), Deep Well Plate 96/1000 µL Eppendorf (top)	

User Intervention – sample addition
For each individual sample, 30 mg of plant material was placed into the provided sample lysis tubes containing the steel ball (C2). The sample tubes are then returned to position C2 on the ep*Motion* system.
Automation
The automated procedure starts with the transfer of the sample tubes from C2 to position TMX; followed by the addition of 180 µL of Lysis Buffer (C1 position 1) to each of the sample lysis tubes (TMX); followed by the addition of 40 µL plant neutralisation buffer (C1 position 2).

User intervention – sample lysis
Lysis tubes are removed from the ep*Motion* and tubes sealed with the caps provided. The samples are fully homogenised using a mixing mill. The tubes are centrifuged at 1500 g for 10 seconds to remove any debris. The sealing caps are removed and the tubes returned to the TMX position on the ep*Motion*.
Automation
The automated protocol continues. A further 260 µL of plant neutralisation buffer (C1 position 2) is added to the sample tube. The sample is mixed by the TMX module at 1000 rpm for 30 seconds at 25 ˚C.

User Intervention – loosen tube caps
Loosen lysis tube caps.
Automation
The automated protocol continues. The samples are incubated in the TMX module for 60 min at 65 ˚C. 335 µL of Magnetic bead & RNase A solution (C1 position 3) are added to the purification block (C4). Note: The magnetic bead suspension is mixed carefully in the reservoir before aspiration to ensure that all of the Magnetic Particles are resuspended.

User Intervention – lysate transferral to purification block
The Lysis tubes and purification block (C4) are removed from the epMotion. The sample tubes are sealed and centrifuged at 1500 g for at least 1 minute to pellet down the unlysed material. 300 µL of the sample lysate is transferred to the purification block taking care to avoid any potential sample cross contamination. The lysis tubes and purification block are returned to the epMotion.
Automation
The sample tubes are transferred to position C2 and the purification block to position TMX. The sample lysates are shaken by the TMX module at 1200 rpm for 5 minutes at 25 ˚C. During this step the DNA binds to the Nucleon magnetic beads. The purification block is transported to the Magnetic separation stand (C4) where the magnetic beads are immobilised. The remaining supernatant is removed followed by the addition of 250 µL of Wash buffer (C1 position 4). The purification block is transported to the TMX module and shaken at 1200 rpm for 3 min at 25 ˚C to allow resuspension of the magnetic beads. The purification block is returned to position C4 and the wash procedure is repeated a further two times. After a drying step to remove residual alcohol the DNA is eluted in 200 µL elution buffer. The purification block is mixed at 1000 rpm for 5 minutes to encourage elution of DNA from the magnetic beads. Following this stage the block is transported to position C4 to immobilise the beads, and the final sample is transferred to the elution plate (C3). The total processing time is approximately 4 hours for 96 samples (3 hours of the processing steps are automated).

Methodology notes:
In the absence of the integrated thermomixer unit the incubation stages can be performed offline and the software amended accordingly. This will require a vortex mixer and an incubator set at 65 ˚C.

Yield and Purity of DNA

DNA yield and purity (260/280 ratio) were determined spectrophotometrically on a Thermo Scientific NanoDrop system. 1.1 µL of neat DNA was analysed.

A subset of samples were further analysed by agarose gel electrophoresis to assess the quality of the genomic DNA. 10 ng of DNA was loaded on a 1 % agarose e-gel. Visualisation was achieved with Ethidium bromide (10 mg/mL). 5 ng lambda DNA was used as a positive control and a buffer only sample applied as a negative control. In addition samples were also subjected to restriction digestion using *Alu* I (5 units incubated with 10 µg of DNA at 37 ˚C for 2 hours) prior to analysis by agarose gel electrophoresis (1 % gel).

Cross contamination Assay

To ensure the automated process delivered precise and reliable pipetting with no well to well cross contamination plant samples were placed in alternate wells to create a checkerboard pattern (Figure 2). The plate was processed and the eluted samples and negative controls were then subjected to DNA quantification and gel analysis as described above.

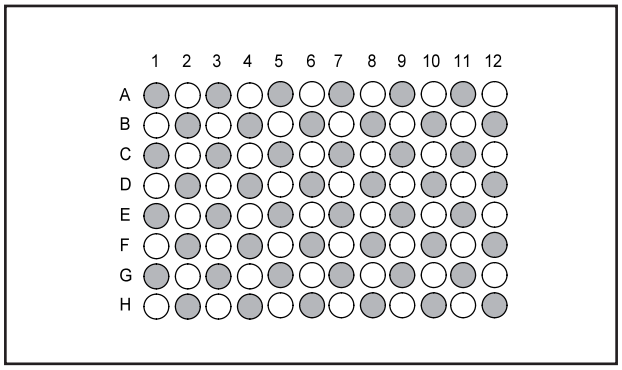

Fig. 2: Cross contamination assay pipetting layout. 48 Plant samples were applied to lysis plate (grey wells). The white wells depict negative controls where no plant material was present.

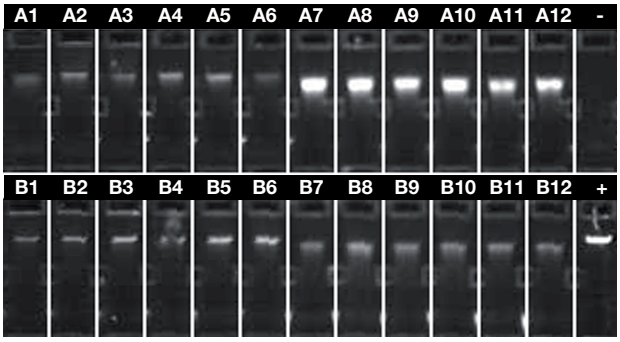

Fig. 3: Agarose gel electrophoresis of purified DNA samples from Cabbage leaves (A1-A6), Pine Needles (A7-A12), Rose leaves (B1-B6) and Corn (B7-B12). – denotes the negative buffer control, + denotes the Lambda DNA positive control.

Results and Discussion

Yield & Purity of DNA

As shown in Table 2 and Figure 3, DNA from various plant samples can be easily purified with the Nucleon Plant DNA Kit and the automated ep*Motion* 5075 TMX system. The method was found to deliver consistently high purity DNA with a mean 260/280 absorbance ratio of 2.05 indicating low protein contamination in the resulting DNA samples. The average total yield across the sample types, derived from 30 mg of starting plant material, was found to be 11.65 µg.

Quality of DNA and Structural Integrity

In order to further demonstrate quality and structural integrity of the isolated DNA the purified DNA samples were analysed by agarose gel electrophoresis (Figure 3.) The cabbage sample was further analysed with and without treatment with restriction enzyme. The automated purification procedure was shown to produce high molecular weight DNA as indicated by the clear bands (Figure 4A). Efficient restriction enzyme digestion was achieved with the gels producing the characteristic smear of DNA (Figure 4B).

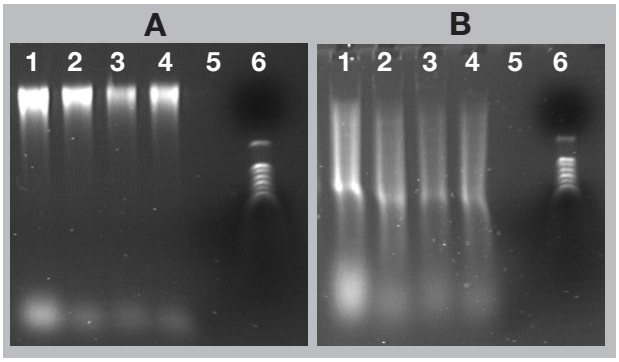

Fig. 4: A. Agarose gel electrophoresis of purified DNA samples from Cabbage leaf without (A) and following (B) restriction digestion. Lanes 1-4 purified cabbage DNA; Lane 5 negative control; Lane 6 DNA ladder.

Cross contamination

Assessment of cross contamination spectrophotometrically indicates that no DNA can be detected from the 48 cabbage leaf sample preparations in the negative control wells (Table 3). This finding was further supported by gel analysis (Figure 5.)

Table 2. Yield and purity of DNA. Variability in sample yield is not unexpected when purifying DNA from plant material, this can usually be attributed to the off-line sample preparation stage.

Sample	Initial sample weight (mg)	n	DNA Purity ($A_{260/280}$)	%CV	Mean DNA concentration (ng/mL)	Mean total yield (mg)	%CV
Cabbage	30	64	2.18	1.7	109.63	21.93	31.9
Pine	30	18	2.00	4.1	26.16	5.23	35.1
Rose	30	18	1.67	2.9	61.84	12.37	13.3
Lettuce	30	18	2.23	6.4	15.96	3.19	20.7
Corn	30	16	2.14	7.1	22.88	4.58	74.1
Barley	30	8	2.06	2.9	54.01	10.80	26.3
	Overall mean purity		**2.05**		**Overall mean yield**	**11.65**	

Table 3: Spectrophotometric assessment of Cross Contamination

Sample	Initial sample weight (mg)	n	DNA Purity ($A_{260/280}$)	Mean DNA concentration (ng/µL)	Mean total yield (µg)	%CV
Cabbage	30	48	2.19	104.88	20.98	33.0
Empty	0	48	0.91	1.12	0.22	-

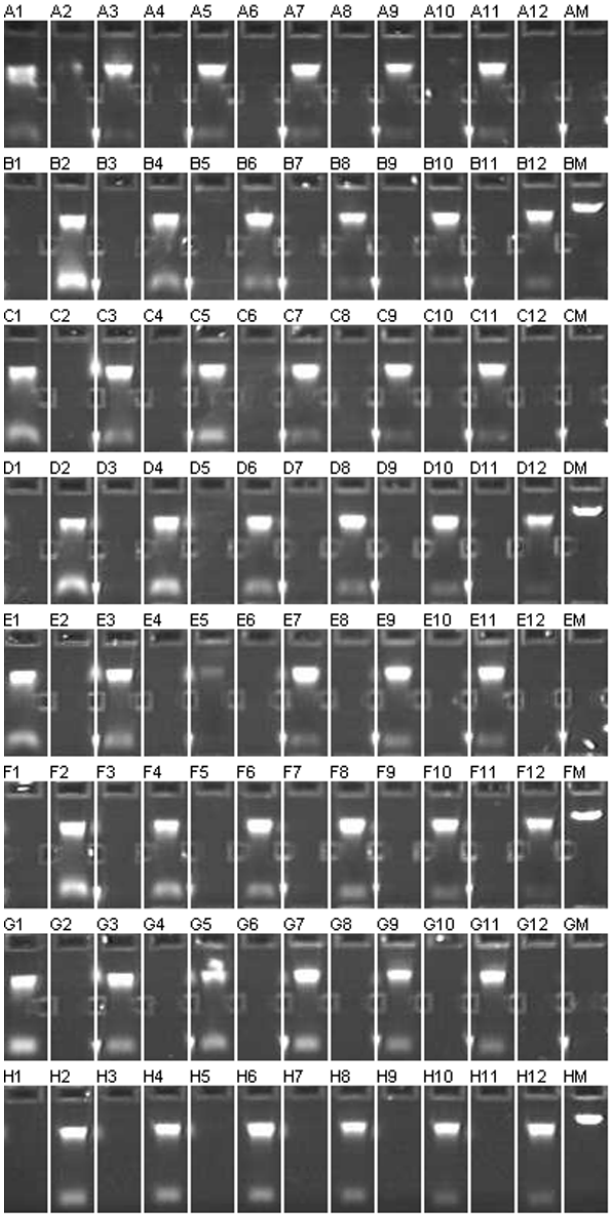

Fig. 5: Evaluation of cross contamination by agarose gel electrophoresis of purified DNA samples from Cabbage leaves processed in alternate wells. – The wells AM, CM, EM and GM are the negative buffer control, BM, DM, FM and HM are the Lambda DNA positive control. RNA contamination is evident in some gels. This may be avoided with a longer incubation with RNase A and bead mixture or increased levels of RNase A.

Conclusion

The integration of the Nucleon Plant DNA Kit with the ep*Motion* 5075 TMX platform provides a reliable, convenient and flexible system for the automated purification of high quality, high yield DNA from up to 96 plant samples in approximately 4 hours with minimal hands on time. This easy to handle system benefits not only from increased throughput, but also reproducible purification of high quality, high molecular weight DNA for use in sensitive downstream applications.

References

[1] Eppendorf operating manual ep*Motion* 5075 TMX
[2] Nucleon Plant DNA Kit user manual

Ordering information

Eppendorf		
Product	**Order No. International**	**Order No. North America**
ep*Motion*® 5075 TMX with PC	5075 000.784	960020444
Dispensing Tool TM 1000-8	5280 000.010	960001010
Gripper	5282 000.018	960002270
Reservoir rack	5075 754.002	960002148
Reservoir 30 mL	0030 126.505	960051009
Reservoir 100 mL	0030 126.513	960051009
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