Applications
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Reproducible and Easy Automated Purification of Plant Genomic DNA

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Abstract

The purification of genomic DNA from plant tissue is required for many methods in general plant molecular biology. In large plant breeding projects or in the analysis of transgenic elements in genetically modified plants many individual samples need to be processed. Here we describe an automated method for the purification of plant genomic DNA consisting of the Promega Wizard® Magnetic 96 DNA Plant System and the Eppendorf liquid handling workstation epMotion® 5075 LH. Various leaf and seed plant samples were used as starting material and the extracted genomic DNA was subjected to PCR analysis. The quality of individual samples was assessed in a cross contamination assay.

Introduction

Many of the common methods to isolate genomic DNA from plant material, for example the CTAB (hexadecyltrimethylammonium bromide) extraction [1], require the use of organic solvents, are labor- and time intensive and can not readily be adapted to robotic platforms. In large scale plant genomic projects the purification of hundreds or thousands of samples often represents a bottleneck in sample analysis. The use of an automated, easy to handle method is therefore highly desirable to increase productivity. If the detection of individual characteristics within many different samples is required, care should be taken that the used automated procedure not only increases sample throughput, but also ensures individual sample quality.

The Promega Wizard Magnetic 96 DNA Plant System provides high quality DNA for downstream applications such as PCR, random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and sequence repeat/short tandem repeat analysis (SSR/STR) [2]. The use of paramagnetic particles (PMPs) that can readily bind the genomic DNA offers consistent yield and ease of handling. The Eppendorf epMotion 5075 LH workstation is a modular robotic workstation that can flexibly be adapted to various liquid handling tasks. The concept of exchangeable tips and the “Free Jet Dispensing” technology enables this system to dispense liquids contact-free and makes it ideal for the execution of complex work steps where high precision and reliability are required [3].

Materials and methods

Equipment

- Eppendorf epMotion 5075 LH with Gripper
- Dispensing tool TM 1000-8, Dispensing tool TM 300-8
- Promega Wizard Magnetic 96 DNA Plant System
- Promega MagnaBot® 96 Magnetic Separation Device
- Two MagnaBot Spacer
- Eppendorf HotMasterMix
- Eppendorf twin.tec PCR Plate 96

Sample material

The following plant starting material was used:

- Tomato leaf *(Solanum lycopersicum)*
- Sunflower seed *(Helianthus annuus)*
- Lettuce *(Lactuca)*
- Carrot leaf *(Daucus carota)*
- Potato tuber *(Solanum tuberosum)*
- Radish leaf *(Raphanus sativus)*
- *Arabidopsis*
- Wheat *(Triticum)*
- Barley *(Hordeum vulgare)*
- Corn leaf *(Zea mays)*
- Rape *(Brassica napus)*
- Onion *(Allium cepa)*.

Sample preparation

Per individual sample 0.15 g plant material was grinded in 300 µl Lysis Buffer A (LBA) with mortar and pestle. The homogenous suspension was transferred to individual 1.5 ml reaction tubes (Eppendorf). 300 µl of LBA was added and mixed well. The samples were centrifuged in

a MiniSpin® centrifuge (4000 rpm, 1073 xg, 10 min, RT) (Eppendorf). For each sample 300 µl of the clear supernatant was transferred to a 96 reaction plate. The plate was then transferred to the epMotion 5075 LH for automated processing.

Alternatively the plant material can be processed using a Geno/Grinder™ 2000, Retsch MM300 Mixer Mill or similar device for more convenient handling of multiple samples. Please refer to the Promega Wizard Magnetic 96 DNA Plant System manual for complete instructions on preparing working solutions.

Automated sample processing with the epMotion 5075 LH

Before starting the method the worktable was prepared according to Figure 1. Detailed information on the required labware for each position on the worktable can be found in Table 1. For 96 samples 6.12 ml MagneSil®/LBB mix solution, 25 ml Wash Buffer and 7.6 ml TE Buffer or nuclease free water was needed. The MagneSil/LBB mixture consists of 0.85 ml Magnetic particles and 5.27 ml Lysis Buffer B (LBB). It was prepared freshly just prior to use.

The automatic procedure starts with transferring 125 µl of each sample to the appropriate wells of the provided u-bottom plate. 60 µl of the LBB/Magnetic Particle mixture are added to each sample. The suspension is being mixed carefully before aspiration to ensure that all of the Magnetic Particles are resuspended. After a 5 minute waiting step that ensures quantitative binding of the DNA, the sample plate is transported to the MagnaBot magnet. The whole supernatant is being removed and two washing steps with 150 µl and 100 µl Washing Buffer follow. After a drying step to completely remove residual alcohol the DNA is eluted in 75 µl TE Buffer. The processing time is approximately 2 hours for 96 samples.

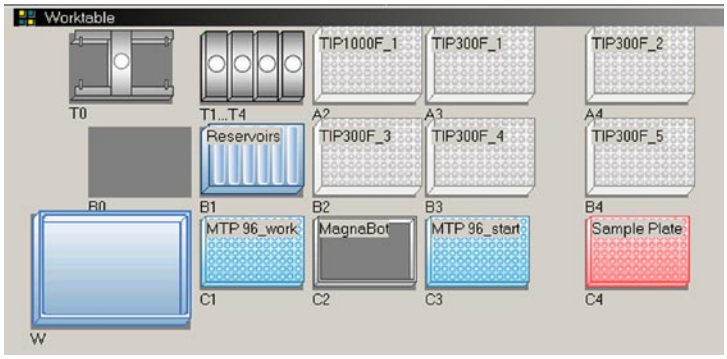

Fig. 1: Screenshot from the epMotion Editor showing the setup of the epMotion 5075 LH worktable for the Magnetic 96 DNA Plant System protocol.

Table 1: epMotion 5075 LH worktable details

Position	Labware	Comment
A2	ep T.I.P.S. Motion 1000 µl	32 tips for 96 samples
A3	ep T.I.P.S. Motion 300 µl	96 tips for 96 samples
A4	ep T.I.P.S. Motion 300 µl	96 tips for 96 samples
B1	Reagent Reservoirs	
	Position 1: Magnetic particles /LBB mixture	30 ml reservoir
	Position 2: Wash Buffer	30 ml reservoir
	Position 3: Elution Buffer	30 ml reservoir
	Position 6: Waste	100 ml reservoir
B2	ep T.I.P.S. Motion 300 µl	96 tips for 96 samples
B3	ep T.I.P.S. Motion 300 µl	96 tips for 96 samples
B4	ep T.I.P.S. Motion 300 µl	96 tips for 96 samples
C1	MTP 96 Plate	Provided in kit
C2	MagnaBot magnetic separation device	equipped with two
C3	MTP 96 Plate	300 µl of starting samples
C4	twin.tec 96 PCR Plate	Elution plate

PCR amplification of TrnL

The samples were analyzed with PCR using the Eppendorf HotMasterMix. For the amplification of the intron of the TrnL chloroplast gene a universal primer pair was used that amplifies the sequence across many plant species [4,5]. The primer sequences can be found in Table 2. The reaction conditions and cycling parameters are listed in tables 3 and 4.

Table 2: Primer sequences used for TrnL amplification

TrnL Forward primer	5´- CGAAATCGGTAGACGCTACG-3´
TrnL Reverse primer	5´- GGGGATAGAGGGACTTGAAC-3´

Table 3: Reaction conditions for TrnL PCR

Component	Volume	Final concentration
Template	2 µl	20 ng genomic DNA
Nuclease free water	18 µl	
Forward Primer	5 µl	200 nM
Reverse Primer	5 µl	200 nM
Eppendorf HotMasterMix	20 µl	1 x
Total reaction volume	**50 µl**	

Table 4: Incubation program for TrnL PCR

Step	Temp.	Duration	Cycles
Initial denaturation	94°C	1 minute	1 x
Denaturation	94°C	1 minute	
Primer annealing	55°C	1 minute	30 x
Elongation	70°C	1 minute	
Final elongation	70°C	1 minute	1 x

Cross contamination assay
A cross contamination assay was performed to assess if the automated process ensures precise and reliable pipetting within each well without affecting adjacent samples. Every second well was filled with water instead of the plant sample to create a chessboard pattern. The pipetting scheme is illustrated in Figure 2. The plate was processed as described above and the eluted samples and negative controls were subjected to PCR analysis as a very sensitive method for detecting any contaminating DNA in the controls.

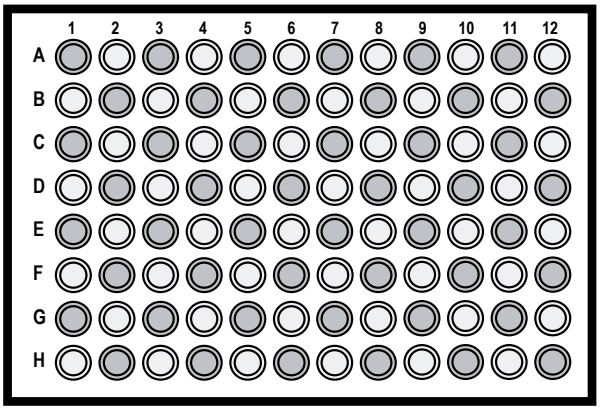

Fig. 2: Pipetting scheme of the cross contamination assay. 48 plant samples were applied to the 96 reaction plates (positions labeled grey). The white positions were filled with water as negative controls.

Results

As illustrated in Figure 3 A genomic DNA from various plant samples can be isolated with the Wizard Magnetic DNA Plant System and the automated epMotion 5075 LH method. The procedure consistently delivered high molecular weight DNA as indicated by clear bands without detectable RNA contamination. The DNA was suitable for PCR amplification which is demonstrated by the succesful amplification of the TrnL sequence in all of the samples (Figure 3 B).Typical yields for different plant source material can be taken from Table 5. For example 0.12 g of onion leaves yielded an average concentration of 37 ng/µl genomic DNA in 65 µl TE Buffer per well equalling a total yield of 2.4 µg DNA.

The results from the cross contamination assay is illustrated in Figures 4 and 5. The total 96 reaction preparation of onion genomic DNA is shown in Figure 4. No visible genomic DNA can be detected in the control wells filled with water. The results of the PCR amplification of the samples are shown in Figure 5. In the control reactions no amplification of the TrnL sequence could be detected.

Table 5: Weight of starting material and typical DNA yield

Leaf starting material (per well)	DNA Concentration	Total yield
Onion, 120 mg	37 ng/µl	2.4 µg
Tomato, 350 mg	28 ng/µl	1.8 µg
Wheat, 280 mg	24 ng/µl	1.6 µg
Rape, 330 mg	71 ng/µl	4.6 µg
Arabidopsis, 250 mg	25 ng/µl	1.6 µg

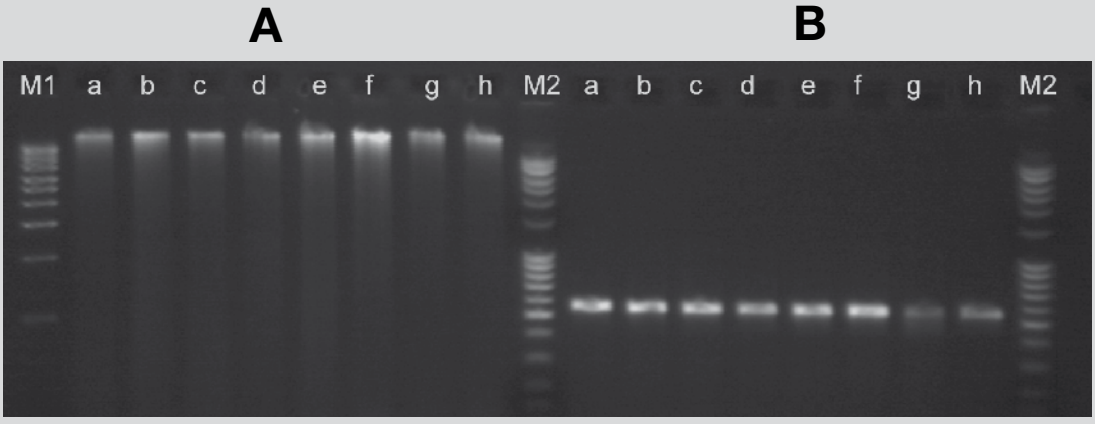

Fig. 3 A: Genomic DNA was isolated from a) Rape, b) Carrot leaf, c) Tomato leaf, d) Wheat, e) Onion, f) Barley, g) Corn leaf, h) *Arabidopsis*.
Fig. 3 B: Amplification of the TrnL sequence from these samples. 20µl of the genomic DNA and 3 µl of the PCR product were run on a 1% agarose gel. M1) 100 bp ladder M2) 100 bp DNA-ladder, extended (Roth).

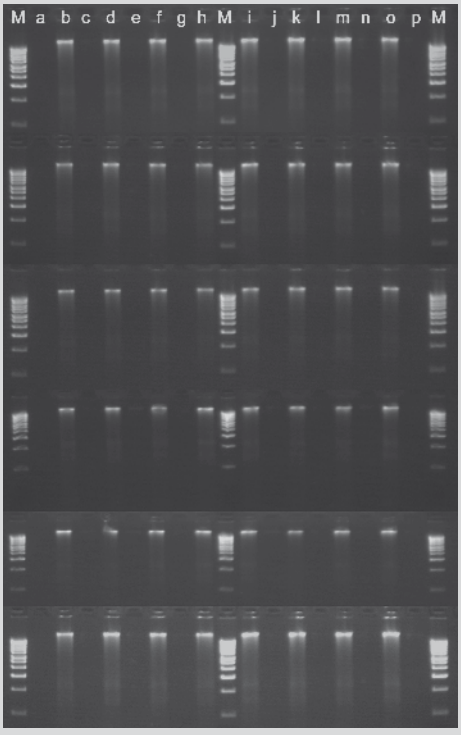

Fig. 4: Cross contamination assay:
Genomic DNA was isolated from Onion.

In the lanes b, d, f, h, i, k, m, o the plant samples were applied in a chessboard pattern. Lanes a, c, e, g, j, l, n, p show the water negative controls. 20 µl of the eluted samples were run on a 1 % agarose gel. The size marker is 100 bp ladder (Roth).

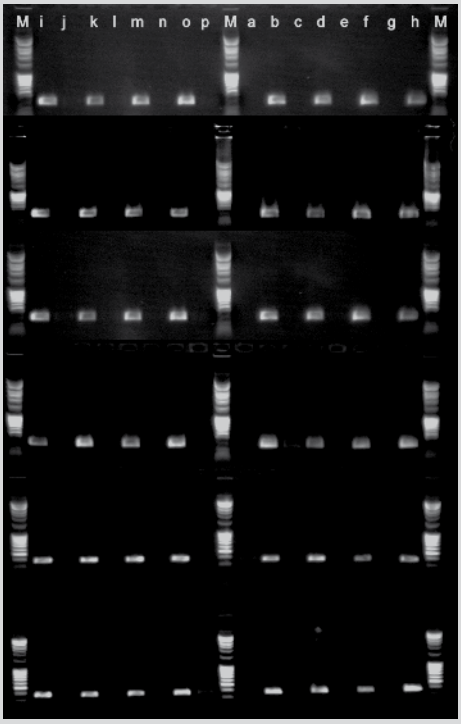

Fig. 5: Cross contamination assay:
PCR amplification of samples and controls.

Lanes i, k, m, o, b, d, f, h, show the TrnL amplification product of the Onion genomic DNA samples. In the lanes j, l, n, p, a, c, e, g the water control gradiens were applied.

Discussion

Purification of plant genomic DNA from multiple samples can be a time consuming and demanding process. The integration of the Promega Wizard Magnetic 96 DNA Plant System into the workstation epMotion 5075 allows the user to automatically extract DNA from 96 plant samples in less than two hours with minimal hands-on time. The procedure is convenient and very easy to perform. Individual liquid handling parameters within the automated method protocol may be adapted to special plant samples easily with the intuitive software structure of the epMotion control panel. In addition to the time-saving and productivity-enhancing aspects of an automated process, the sample quality and reliability is an equally important factor, especially if genetic differences between plant samples are detected with sensitive downstream applications. The absence of PCR amplification from control wells in the performed cross contamination assay shows that the sample preparation process on the epMotion 5075 can fulfill these requirements.

References

Literature
[1] Doyle, J.J. and Doyle, J.L. (1987) Phytochem. Bull. 19, 11-15
[2] Promega Notes (2001) Number 79
[3] Apostel, F. (2003) Eppendorf BioNews 20, 1-2
[4] Taberlet, P. et al (1991) Plant Mol. Biol. 17, 1105-1109
[5] Whittle C.-A. et al (2002) Mol. Biol. Evol. 19(6), 938-949

Protocols
Instrument Manual for the epMotion 5075 LH, Eppendorf AG
Wizard, Magnetic 96 DNA Plant System, Technical Bulletin #TB289, Promega Corporation

Ordering information Eppendorf

Product	Description	Order no. International	Order no. North America
epMotion® 5075 LH 230 V		5075 000.008	n/a
epMotion® 5075 LH 120 V		n/a	960020006
Dispensing tool TM 1000-8		5280 000.258	960001061
Dispensing tool TM 300-8		5280 000.231	960001052
Gripper		5282 000.018	960002270
Holder for gripper		5075 759.004	960002211
Reservoir Rack		5075 754.002	960002148
Reservoirs 100 ml	(10 x 5 reservoirs in bags/case, PCR clean)	0030 126.513	960051017
Reservoirs 30 ml	(10 x 5 reservoirs in bags/case, PCR clean)	0030 126.505	960051009
epTIPS Motion 1000 µl Filter		0030 003.993	960050100
epTIPS Motion 300 µl Filter		0030 003.977	960050061
twin.tec PCR Plate 96, skirted, green	0030 128.664	951020443	
HotMasterMix (2,5x), 1000 units		0032 002.722	954140199

Ordering information Promega

Product	Order no.
Wizard® Magnetic 96 DNA Plant System (4x96 reactions)	FF3761
Magnabot® Magnetic Separation Stand	V8151
Magnabot® Spacer	V8381

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