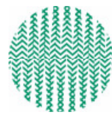

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Application Note

Application Note

Semi-automated genomic DNA extraction from Plant with CyBi®-SELMA 96/1000 µl using NucleoSpin® 96 Plant II Kit

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Key words: CyBi®-SELMA, MACHEREY-NAGEL NucleoSpin 96 Plant II kit, Plant genomic DNA extraction, High throughput

Abstract

The CyBi®-SELMA 96/1000 µl was used to automate MACHEREY-NAGEL NucleoSpin 96 Plant II technology for plant genomic DNA extraction. The use of a 96-well pipetting head enables a rapid automated procedure while maintaining the purification technology's highly reliable performance. The result is good yields (1-8 µg species function) of highly purified DNA (A260/280 > 1.8).

Introduction

This extraction method is based on the established CTAB and SDS lysis metods (1). As plants are very heterogenous and contain many different metabolites like polyphenols, polysaccharides, or acidic components, NucleoSpin® Plant II offers two different lysis procedures for optimal processing of various samples. We used the PL1 lysis Buffer based on the CTAB lysis method. In addition, the silica membrane in the NucleoSpin® Plant II Columns is optimized to improve DNA binding, and NucleoSpin® Filters are included for convenient clarification of lysates.
NucleoSpin® Plant II allows processing of up to 100 mg (wet weight) or 20 mg (dry weight) starting material, with typical yields in the range from 1 to 8 µg DNA. The eluted DNA is ready to use for PCR reactions.

The CyBi®-SELMA 96/1000µl is a semi-automatic parallel pipettor which works fast, precisely and in a high reproductive manner. Since pipetting to 96 wells simultaneously is advantageous for automating NucleoSpin® 96 Plant II kit, the SELMA was evaluated for its ability to speed up plate processing compared to using an 8-tip manual pipettor.

***Figure1**: The CyBi®-SELMA 96/1000 µl with deep well tips and 2 working positions*

Materials and Methods

The MACHEREY-NAGEL NucleoSpin® 96 Plant kit extraction protocol was set up on a CyBi®-SELMA 96/1000 µl system consisting of a 2 position deck equipped with a 96-tip pipetting head and 1000 µl disposable tips. The purification procedure was established according to MACHEREY-NAGEL's standard protocol for NucleoSpin® 96 Plant II kit. The protocol was carried out with 100 mg freshly plant leaves and 3 mm glass beads (5 per well), lyophilized with a freeze dryer and mixed with a shaker. Purity and yield of extracted DNA was determined by measurement of OD at 260 nm and 280 nm and by calculating the A260/280 ratio.

Devices

- CyBi®-SELMA 96/1000 µl
- Shaker/Gyromixer SO-20a Fluid Management
- Centrifuge Eppendorf 5810R (C1)
- Centrifuge Beckman Coulter Allegra X-15R (C2)
- PherastarFS (BMG Labtech)
- Freeze Dryer (LABCONCO)

Reagents

- MACHEREY-NAGEL NucleoSpin 96 Plant II kit incl. NucleoSpin® Plant II Binding Plates, cap strips, buffers, RNase A (# 740468.4)

Consumables

- CyBi-Tip Rack 96/ 1000 µl DW (CyBio # OL3811-25-539-N)
- Reagent strip reservoir 30 ml (CyBioFrance # 2320230-00)
- 96 Deep well plates 2.2 ml (ABgene # AB0932)
- 96 Deep well plates 1.2 ml (ABgene # AB0564)
- 96 Deep well plates 0.8 ml (ABgene # AB0765)
- 96 Deep well plates 370 µl (ABgene # AB0796)
- Heat seal film (thermal bond, 4titude # 4ti-0591)

Semi-automated procedure

The NucleoSpin Plant II Genomic DNA extraction steps using CyBi-SELMA 96/1000 µl are summarized in Tab.1.

1. Place buffer PL1 with 2% RNAse A in a reservoir on position 1 and sample plate on position 2 of the CyBi®-SELMA deck as shown in Figure 2.
2. Transfer 500 µl of buffer PL1 with 2% RNase A from reservoir at position 1 to sample plate at position 2. Close tubes with heat seal film. Mix vigorously for 15-30 s using a shaker. Spin briefly for 30 s at 1,500 x g (C1) to collect any sample. Incubate samples at 65 °C for 30 min. Centrifuge the samples for 20 min at full speed (5,600-6,000 x g, C2). Remove seal film.

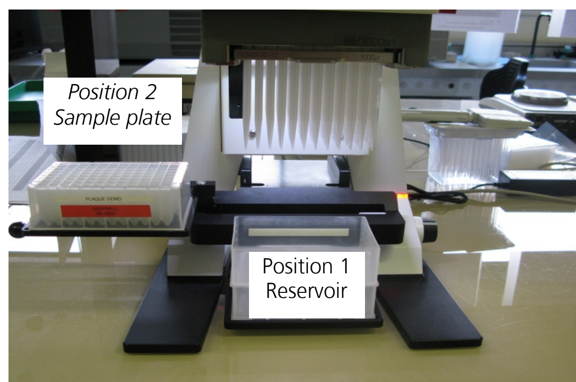

***Figure2:** CyBi®-SELMA 96/1000 µl process layout for semi-automation of the NucleoSpin® 96 Plant II Kit: reagent transfer*

3. Pipette 450 µl Binding Buffer PC from a reservoir at position 1 to a 96 deep well plate 1.2 ml at position 2.
4. Place the cleared lysate plate from step 2 in position 1. Pipette 400 µl cleared lysate sample to deep well plate at position 2 and mix at least 15 times with 500 µl mixing volume.
5. Place NucleoSpin® Plant II binding plate on a 96 deep well plate 2.2 ml. Dispense 800 µl sample into NucleoSpin® Plant II Binding Plate with CyBi®-SELMA as shown in Figure 3.

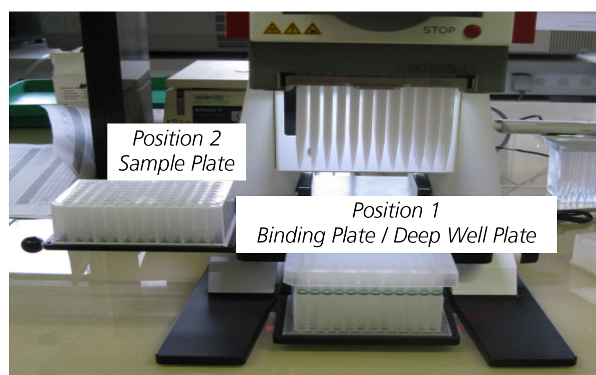

***Figure3:** Process layout for semi-automation of the NucleoSpin® 96 Plant II Kit: binding step*

6. Centrifuge the NucleoSpin® Plant II binding plate stacked on a 96 deep well plate 2.2 ml at 5,600-6,000 x g for 7 min (C2).

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***Table 1**. NucleoSpin Plant II Genomic DNA purification liquid handling steps using CyBi®-SELMA 96/1000 µl*

Step	Labware SELMA Position 1	Labware SELMA Position 2	Action	Pipetting Mode
Cell lysis	Reagent reservoir buffer PL1 with 2%RNase A	Sample plate (deep well plate 2.2 ml)	Pipette 500 µl buffer PL1 with 2% RNase A into sample plate	Pipetting
Binding buffer transfer	Reagent reservoir buffer PC	Deep well plate 1.2 ml	Pipette 450 µl binding buffer PC into deep well plate	Pipetting
Sample transfer	Sample plate	Deep well plate 1.2 ml with buffer PC	Pipette 400 µl clear lysate into deep well plate 1.2 ml, mix 15 x (500 µl)	Pipetting, Mixing
Binding DNA	Deep well plate 1.2 ml from step above	NucleoSpin® Plant II binding plate stacked on a deep well plate 2.2 ml	Pipette samples into NucleoSpin Plant II binding plate	Pipetting
Wash 1	Reagent reservoir buffer PW1	NucleoSpin Plant II binding plate stacked on a deep well plate 2.2 ml	Pipette 400 µl PW1 into binding plate	Pipetting
Wash 2	Reagent reservoir buffer PW2	NucleoSpin Plant II binding plate stacked on a deep well plate 2.2 ml	Pipette 700 µl PW2 into binding plate	Pipetting
Wash 3	Reagent reservoir buffer PW2	NucleoSpin Plant II binding plate stacked on a deep well plate 2.2 ml	Pipette 700 µl PW2 into binding plate	Pipetting
Elute DNA	Reagent reservoir buffer PE	NucleoSpin Plant II binding plate stacked on a deep well plate 370 µl	Pipette 130 µl pre-warmed buffer into binding plate	Pipetting

Semi-automated procedure ...

7. Pipette 400 µl PW1 to the NucleoSpin® Plant II Binding Plate with CyBi®-SELMA as shown in Figure 3.
   Seal plate and centrifuge again at 5,600–6,000 x g for 2 min. Place NucleoSpin® Plant II Binding Plate on 96 deep well plate 2.2 ml.
8. Pipette 700 µl PW2 to the NucleoSpin® Plant II Binding Plate with CyBi®-SELMA as shown in Figure 2. Seal plate and centrifuge again at 5,600-6,000 x g for 2min.
9. Repeat step 8 and place NucleoSpin® Plant II Binding Plate on a new 96 deep well plate 370 µl.
10. Pipette 130 µl pre-warmed Buffer PE (70 °C) to the NucleoSpin® Plant II Binding Plate with CyBi®-SELMA as shown in Figure 2. Dispense the buffer directly onto the membrane and incubate at room temperature for 2 min.
11. Centrifuge at 5,600-6,000 x g for 2 min and remove the NucleoSpin® Plant II Binding plate.

Results and Discussion

Purified genomic DNA with high quality was obtained. The DNA was successfully used for a PCR amplification with fluorescent labeled primers (Figure 4). Amplification of the expected PCR fragment indicated the absence of PCR inhibitors. DNA yield was calculated at 5 µg from 100 mg plant. Purity was determined by measurement of OD 260 nm/ 280 nm and calculated to be 1.8.

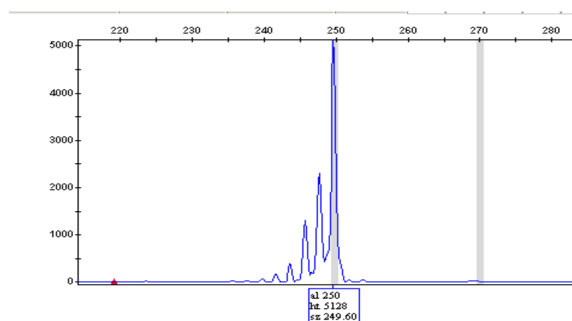

***Figure 4**: PCR amplification of SSR marker fragment (250bp) after electrophoresis on 3130 xl Genetic Applied Biosystem*

The semi-automated method described above successfully performed DNA isolation from leaf tissue and delivered good results in terms of DNA yield and quality. Outstanding features of the CyBi-SELMA are the high-throughput of the system with short pipetting time, the easy handling of the robot, the high flexibility of the liquid handling method settings and the small size allowing its use under a sterile hood.

In comparison, before we used a fully automated robot equipped with 3 x 8 1000 µl channels and in one working day (8 hours) only 4 x 96 DNA extractions were possible. With the CyBi®-SELMA 96/1000 µl we realized 8 x 96 DNA extractions in the same 8 hour day, and we can use it under a sterile hood.

Conclusion

The results demonstrate that the semi-automated CyBi®-SELMA 96/ 1000 µl pipettor is very well suited for reliable automation of 96-well based genomic DNA extraction from plants using NucleoSpin® 96 Plant II kit technology. 8 x 96 high quality DNA extractions can be performed in one 8 hour working day. The small footprint of the CyBi®-SELMA allows a convenient handling under a sterile hood. Results are excellent and anyone can use it without to be a programming-robot expert.

References

(1) Moeller E. M., Bahnweg G., Sandermann H. and Geiger G. G. (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids research* **20**: 22, 6115-6116.

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