

Application Note

Automated Genomic DNA Extraction from Plants with the CyBi[®]-RoboSpense using the MACHEREY-NAGEL NucleoSpin[®] 96 Plant Kit

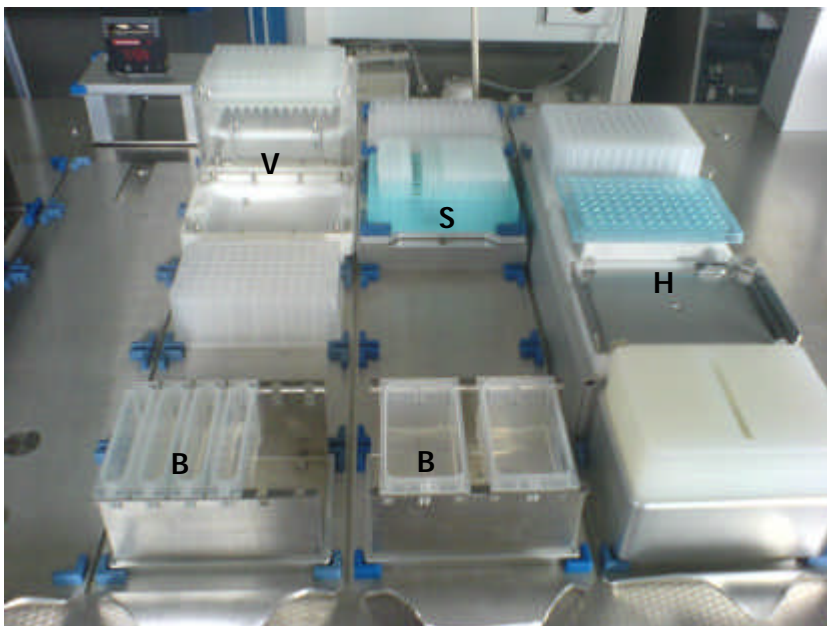
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Abstract: The MACHEREY-NAGEL NucleoSpin[®] 96 Plant Kit technology for purification of genomic DNA from plants has been automated on the CyBi[®]-RoboSpense. The vacuum filtration accessories, robotic microplate handler and shaker for microplates allowed performing an automated extraction procedure. DNA from wheat samples was extracted with very good yields (7 µg DNA from 50 mg wheat leaves) and excellent purity (OD 260 nm/280 nm=1.7). Extracted gDNA is suitable for downstream applications (PCR).

Introduction: The NucleoSpin[®] 96 Plant kit for isolation of genomic DNA uses a combination of CTAB extraction and subsequent binding to a special silica membrane.

First the plant samples are mechanically disrupted and lysed with a CTAB based Lysis Buffer. The resulting clear lysates are mixed with Binding Buffer and ethanol in order to adjust conditions to enable reversible binding of DNA to the silica membrane of the Binding Plate. After subsequent washing and drying steps, highly pure genomic DNA is eluted with elution buffer or water. This vacuum based purification protocol was automated using the CyBi[®]-RoboSpense.



Picture 1: Process layout for automation of the NucleoSpin[®] 96 Plant Kit

- B** buffers
- H** electronically temperature controlled unit
- S** shaker for microplates
- V** vacuum manifold

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Methods: The MACHEREY-NAGEL NucleoSpin® 96 Plant kit (2x96 preps, MN Cat. No. 740661.2) extraction protocol was set up on a CyBi®-RoboSense system consisting of a 35 position deck equipped with 8 liquid handling channels and disposable tips, 8 high precision pumps with a volume range from 1 to 1000 µL, a vacuum manifold, a shaker for microplates, a robotic microplate handler and an electronically temperature controlled board. The purification procedure was established according to MACHEREY-NAGEL's standard protocol for NucleoSpin® 96 Plant kit. The protocol was carried out with 50 mg freshly homogenized wheat leaves (*Triticum aestivum*) per well.

The quality and integrity of the DNA obtained was analysed by gel electrophoresis and ethidium bromide staining. Yields were calculated by DNA quantification with SYBR® Green I. Purity of extracted DNA was determined by measurement of OD at 260 nm and 280 nm with a SpectraMax® 250 microplate reader and by calculating the $A_{260/280}$ ratio. For an additional quality check, PCR amplification of a gene fragment of NADH-dehydrogenase (831 bp) was performed with 35 PCR cycles.

Automated extraction procedure:

1. Place reagents, tips, samples, and buffers on the CyBi®-RoboSense deck as shown in picture 1.
2. Manually homogenize plant material in Lysis Buffer (e.g., 50 mg of fresh wheat leaves in 500 µL) in a Dispomix® (BioLab Products GmbH) for 7.5 min at 2500 rpm. (Other suitable homogenization tools may also be used.)
3. Add 400 µL Lysis Buffer C1 including RNase to the homogenization tubes containing 50 mg plant tissue. Shake for 30 sec at 1000 rpm and incubate for 30 min at 65 °C.
4. Manually clear lysate by centrifugation for 10 min at 3,500xg.
5. Premix Binding Buffer C4 (300 µL) and ethanol (200 µL) and add 500 µL of the mixture to 300 µL of the cleared lysate. Mix by shaking for 30 sec at 1000 rpm.
6. Transfer the mixture to the NucleoSpin® Plant Binding plate followed by a 2 min incubation.
7. Bind DNA to the NucleoSpin® Plant Binding plate by applying vacuum for 1 min at 900 mbar.
9. Wash the silica membrane three times by adding 500 µL Wash Buffer CW and 2x 900 µL Wash Buffer C5, followed by application of vacuum for 1 min at 600 mbar.
10. Dry the membrane using the automated column drying mode with vacuum for a minimum of 8 min.
11. Elute purified DNA by adding Elution Buffer CE, 75 µL for the first elution step, and 125 µL for a second elution step. After a 1 min wait, vacuum was applied for 1 min at 600 mbar for both steps.

Results: Purified genomic DNA with high quality was obtained. The DNA was successfully used for a downstream PCR amplification (Figure 1). Amplification of the expected PCR fragment indicated the absence of PCR inhibitors. DNA yield was calculated at 7 µg from 50 mg plant (n=16). Purity was determined by measurement of OD 260 nm/280 nm and calculated to be 1.7.

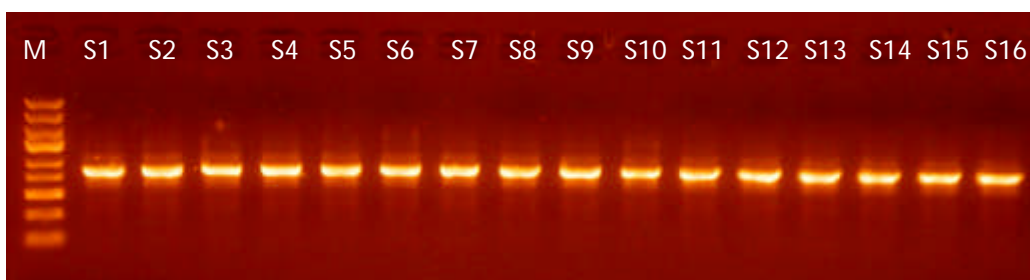


Figure 1: PCR amplification of NADH-dehydrogenase gene fragment (831 bp). Analysis of extracted DNA for their usability in the downstream application PCR. A 831 bp long NADH- dehydrogenase gene fragment was amplified by PCR with 35 cycles and analyzed by gel electrophoresis. Lane M: 6 µL GeneRuler™ Express DNA ladder (Fermentas); lanes S1 -S16 : 10 µL of PCR reaction.



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Discussion: The specialized accessory modules, such as the shaker for microplates and the vacuum filtration unit, made it possible to implement the automated process on the CyBi®-RoboSpense with excellent results.

By shaking the square-well microplate at 1000 rpm on CyBio's shaker for microplates, complete mixing of homogenized plant material with buffer C1 and of cleared lysates with buffer C4 was achieved. This resulted in a decrease of process time and tip consumption because resuspension by pipetting up and down after adding lysis, binding, wash or elution buffer wasn't necessary.

Precise adjustment of tip heights for dispensing in the cavities of the Plant Binding plate helped to avoid contact with the membranes and thus decreasing genomic DNA yields.

Careful adjustment of pipetting speeds, implementation of post-aspiration and -dispension delays (2000 ms), and the use of air gaps ensured reliable transfer of the viscous crude lysates to the NucleoSpin® Plant Binding plate.

Using the unique CyBio® EluteControl software in combination with an electronically controlled pump, the vacuum could be set specifically for every vacuum step as required by the application.

The CyBio® EluteControl software evaluation mode enabled the user to toggle between execute and edit modes during a run. Thus, for example membrane washing was controlled visually in the evaluation mode. Then the vacuum step was repeated with modified settings until the results were satisfactory. The updated values were stored and finally the automated procedure was continued to completion.

Two DNA elution steps were performed, first with 75 µL and in a second step with 125 µL elution buffer at 600 mbar to assure complete elution of DNA and thus increased yields.

Conclusion: The results demonstrate that the CyBi®-RoboSpense is very well suited for reliable automation of vacuum-based genomic DNA extraction from plants using MACHERY-NAGEL's NucleoSpin® 96 Plant kit technology. Using its specialized accessories for vacuum filtration, shaking and temperature incubation as well as the optimization of pipetting parameters and vacuum settings, enabled complete automation of the assay with excellent results.