

# Fully Automated Purification of Genomic DNA from Plant Using NucleoSpin® 96 Plant on the Xiril X100 Platform with Vacuum Filtration

High-throughput extraction of high molecular-weight genomic DNA is the first and often limiting step when processing large numbers of different plant samples. In the fields of plant research and crop design, high reproducibility in yield and quality is mandatory for an optimal performance of downstream processes like genotyping or PCR-based applications e.g. SNP analysis.

The automated extraction system described here meets all requirements for a robust and user-friendly procedure. Manual interactions are minimized due to automated liquid handling, robotic plate handling, integrated shaking, heating, and vacuum filtration. Optionally, initial sample homogenization can also be automated.

With the NucleoSpin® 96 Plant kit excellent DNA yields and outstanding quality of DNA from plant material is achieved. The extracted DNA is suitable for common downstream applications like restriction analysis, Southern Blotting, or PCR.

## Features:

- Fully automated (after homogenization of plant material).
- Flexibility: gDNA from up to 80 mg plant material.
- Fast: 100 minutes for 96 samples (not including lysis).
- Silica-membrane technology.
- Elution volume: 75 – 200 µl.
- High yields: up to 30 µg DNA from plant tissue.
- High consistency: typical CVs for yield are <15%.
- High DNA quality: fragment size up to 30 – 50 kbp, purity  $A_{260/280}$  1.8 – 2.0.
- Efficient removal of PCR inhibitors.

## Instrumentation:

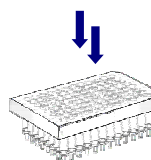
The automation system consists of a Xiril X100 Robotic Workstation equipped with a plate gripper tool, a thermo shaker and solid phase extraction (SPE) unit for vacuum filtration (Fig. 1). The MACHEREY-NAGEL NucleoSpin® 96 Plant kit was used for high yield and high quality DNA extraction. Sample homogenization was performed using the Dispomix homogenization instrument (stand-alone device or integrated on Xiril platform). Alternatively, homogenization can be performed using Genogrinder or Mixer Mill.



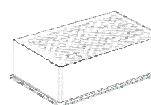
**Fig. 1:** Xiril X100 Automated Liquid Handling System equipped with gripper tool and Solid Phase extraction unit.



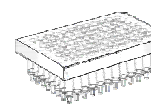
Homogenize plant leaves using the Dispomix instrument



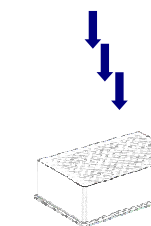
Incubate the homogenate and transfer to Lysate Clearing Plate



Adjust binding conditions, transfer into NucleoSpin® Binding Plate



Bind, wash and elute highly pure gDNA

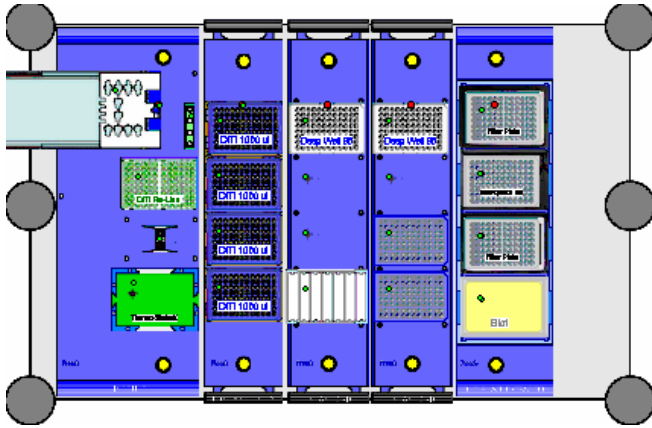


**Fig. 2:** NucleoSpin® 96 Plant procedure with optional lysis clearing plate. For details on the procedure see below.

## Method:

This application note describes the procedure using the Dispomix homogenization instrument and the use of a lysate clearing plate (see Fig. 2). Samples of lyophilized wheat leaves (corresponding to approx. 80 mg wet weight plant material) were homogenized using the Dispomix in lysis buffer C1 supplemented with RNase. For heat incubation 400 µl of each sample were transferred to a square-well block and incubated with shaking for 30-60 minutes at 56°C. Crude lysates (350 µl) were transferred to a lysate clearing plate, overlaid with 200 µl ethanol and filtered into a square-well block. Binding buffer C4 (300 µl) was added to the cleared lysate. After mixing and transferring to the NucleoSpin® Plant Binding Plate the genomic DNA is bound to the silica membrane upon vacuum filtration. After washing with high-salt buffer CW (600 µl) and ethanolic buffer C5 (2x900 µl) the silica membrane of the NucleoSpin® Plant Binding Plate is dried under vacuum for 10 minutes. Highly pure gDNA is then eluted in two steps (100 µl each step) with Elution Buffer CE, prewarmed to 70°C to increase the yield of genomic DNA. The complete procedure is fully automated and the DNA is ready-to-use for common downstream

applications. The deck layout for the Xiril X100 instrument is shown in Fig 3.

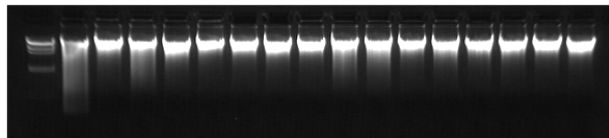


**Fig. 3:** Deck layout of Xiril X100 for isolation of genomic DNA with NucleoSpin® 96 Plant kit using optional lysate clearing plate.

Alternatively to the procedure described above the NucleoSpin® 96 Plant kit can be used without lysate clearing plate. In this case, homogenization is performed either by using the Dispomix or other bead based grinding tools. Following heat incubation the lysate is cleared by centrifugation in order to remove debris. After addition of binding buffer and ethanol to the cleared lysate DNA is bound to the NucleoSpin® 96 Plant Binding Plate. All further steps (washing, elution) are performed as described above.

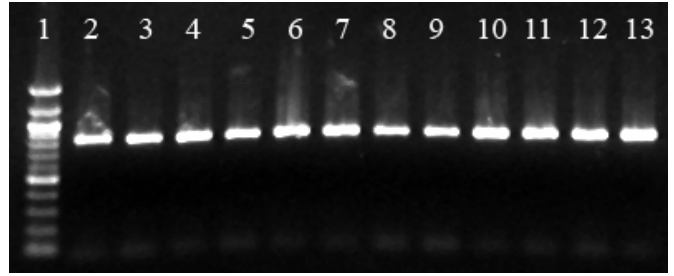
**Results:**

High consistency of yield and quality are shown in Fig 4. The NucleoSpin® 96 Plant kit achieved yields of  $10.77 \pm 1.38 \mu\text{g gDNA}$  from 80 mg wheat leaf. The purified gDNA is ready to be used in common downstream applications. Results of a downstream PCR are shown in Fig. 5



**Fig. 4:** Reproducible yield and quality of genomic DNA from plant.

Genomic DNA was isolated from 50 mg of frozen wheat leaf tissue ( $n=16$ ) according to the standard procedure of NucleoSpin® 96 Plant. From each purified sample 10  $\mu\text{l}$  were loaded on a 0.8 % agarose gel. Size standard:  $\lambda\text{HindIII}$ . High molecular weight DNA was obtained.



**Fig 5:** PCR amplification of NucleoSpin® 96 Plant purified DNA - comparison of homogenization tools.

Randomly selected samples from different purifications were used for PCR amplification. Lane 1: 100 bp size standard; Lane 2-9: samples homogenized with a standard mixer (Bamix, Frauenfeld, Switzerland); Lane 10-13: samples homogenized with Dispomix. PCR products were amplified in 35 cycles from 2  $\mu\text{l}$  of each eluate using primers for *nad5* gene (NADH dehydrogenase, conserved mitochondrial multicopy gene). PCR product from 10  $\mu\text{l}$  of each PCR assay was separated on an agarose gel. The expected PCR product is approximately 750 bp. A specific PCR fragment was obtained from all purified DNA samples indicating the absence of PCR inhibitors.

**Conclusion:**

Combining MACHEREY-NAGEL's NucleoSpin® 96 Plant chemistry with Xiril Robotic Workstations and the Dispomix homogenization tool provides a reliable and flexible method for the automated DNA extraction from plant leaf tissue. The solution eliminates human errors and minimizes the risk of cross-contamination. The automation of the process replaces labor intensive manual protocols, and saves the user's time. Only minimal manual interaction is required due to optional integration of automated homogenization, heat incubation, and lysate clearing.

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**Ordering Information:**

Product	Preps	Cat. No.
NucleoSpin® 96 Plant	2 x 96	740 661.2
NucleoSpin® 96 Plant	4 x 96	740 661.4
NucleoSpin® 96 Plant	24 x 96	740 661.24

**For more information regarding the automated use of MN products, please contact your local representative or visit MN directly under [www.mn-net.com](http://www.mn-net.com).**

Trademarks: NucleoSpin is a registered trademark of MACHEREY-NAGEL

**MACHEREY-NAGEL**



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