

MultiPROBE® II Application Note

Fully Automated Purification of Genomic DNA using the NucleoSpin® 96-well System and the MultiPROBE II HT EX Automated Liquid Handling System with Integrated Robotic Centrifuge

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Abstract

The high-throughput extraction of high molecular-weight genomic DNA with reproducibility in yield and quality is the first and often limiting step in the processing of a broad range of different tissue and plant samples in the field of transgenics, genotyping, PCR-based applications like SNP analysis or agricultural crop science. The system presented here meets the requirements of user-friendly processing without manual interaction (including the lysis step by heat incubation), excellent DNA yields and outstanding quality of DNA from animal or plant tissue. The DNA extracted is suitable for common downstream applications like restriction analysis, Southern Blotting and PCR.

Introduction

The integrated automation solution consisting of the MultiPROBE® II HT Liquid Handling System and Gripper™ Integration Platform with Hettich Robotic Centrifuge and the MACHEREY-NAGEL NucleoSpin® 96-well System based on silica-membrane technology allows the fully automated purification of genomic DNA from animal and plant tissues. The integrated solution is a highly robust system and provides consistency of performance for a variety of different samples types. This application note concentrates on two protocols, the first is Genomic DNA extraction from animal tissue and the second is Genomic DNA extraction from plant tissue.

Experimental

Protocol for 96-well preparation of genomic DNA from animal tissue (e.g. mouse tail clippings) using the MACHEREY-NAGEL NucleoSpin 96 Tissue HC kit (High Capacity) on the PerkinElmer MultiPROBE II HT Automated Liquid Handling System with Gripper Integration Platform, integrated Robotic Centrifuge (Rotanta 46 RSC centrifuge, Hettich, Germany), Microplate Shaker, and Incubator (see figure 1.):

- 1.) *Prepare samples of animal tissue:* For each preparation, cut up to two pieces of maximum 0.5 cm of mouse tail tip section (10 mg) into one well of a Round-well Block (lysis block). If preparing DNA from rat tails, one 0.6 cm piece is sufficient. Place lysis block at the indicated position on the deck of the MultiPROBE II.
- 2.) *Lyse samples:* 180 µl of buffer T1 premixed with 25 µl of proteinase K solution is dispensed to each well of the lysis block. The lysis block is covered. After incubation at 56 °C for 20 minutes, samples are mixed vigorously for 10 minutes on the microplate shaker. After complete lysis of mouse tails (at least 6-8 h or overnight) residual tissue/debris is settled by centrifugation at 5,600 x g for 10 minutes. Additional digestion of cellular RNA by RNase A is optional.
- 3.) *Bind genomic DNA to silica membrane:* 400 µl of buffer BQ1/ethanol (1/1, vol/vol) and 200 µl of lysed sample are aspirated using disposable tips. This solution is transferred to the NucleoSpin Tissue Binding Plate which is stacked on top of an MN Square-well Block (waste container) and mixed by pipetting up and down with disposable tips. Binding of genomic DNA to the silica membrane is achieved during centrifugation at 5,600 x g for 5 minutes.
- 4.) *Wash:* 500 µl of high-salt buffer BW are dispensed to each well of the NucleoSpin Tissue Binding Plate followed by centrifugation at 5,600 x g for 5 minutes (1st wash). 2nd wash is done with 700 µl of ethanolic buffer B5, accordingly. 3rd wash is optional by repeating 2nd wash. A new MN Square-well Block (waste container) is used for the 2nd and 3rd wash.
- 5.) *Dry:* Complete drying of the NucleoSpin Tissue Binding Plate (silica membrane) is achieved by centrifugation at 5,600 x g for 10 minutes during the last wash step.

6.) *Elute highly pure genomic DNA:* The NucleoSpin Tissue Binding Plate is stacked on a Round-well Block (low). 100 µl of buffer BE (prewarmed to 70 °C to increase the yield of genomic DNA) are dispensed to each well of the plate and incubated on the membrane for 2 minutes. Genomic DNA is eluted by centrifugation at 5,600 x g for 5 minutes. Optionally, a second elution step is performed. DNA is ready-to-use for common downstream applications.

Protocol for 96-well preparation of genomic DNA from plant tissue using the NucleoSpin 96 Plant HC kit on the MultiPROBE II HT Automated Liquid Handling System with Gripper Integration Platform, integrated Robotic Centrifuge, Microplate Shaker, and Incubator:

- 1.) *Prepare samples of plant tissue:* For each preparation, collect 50-100 mg of plant material per tube of the MN Tube Strips (lysis block). Add one 4 mm stainless steel bead per well. Close the tubes. Freeze samples in liquid nitrogen. Homogenize samples by vigorous shaking with a suitable homogenization tool (e.g., Genogrinder, Mixer Mill). Place lysis block at the indicated position on the deck of the MultiPROBE II.
- 2.) *Lyse samples:* 400 µl of buffer C1 premixed with 10 µl of RNase A (10 mg/ml) are dispensed to each well of the lysis block. Samples are mixed vigorously for 5 minutes on the microplate shaker. After incubation at 56 °C for 30 minutes, samples are mixed vigorously again. Residual tissue/debris are settled by centrifugation at 5,600 x g for 10 minutes.
- 3.) *Bind genomic DNA to silica membrane:* 500 µl of buffer C4/ethanol (3/2, vol/vol) and 300 µl of cleared lysate are aspirated using disposable tips. This solution is transferred to the NucleoSpin® Plant Binding Plate which is stacked on top of an MN Square-well Block (waste container) and mixed by pipetting up and down with disposable tips. Binding of genomic DNA to the silica membrane is achieved by centrifugation at 5,600 x g for 5 minutes.
- 4.) *Wash:* 500 µl of high-salt buffer CW are dispensed to each well of the NucleoSpin Plant Binding Plate followed by centrifugation at 5,600 x g for 5 minutes (1st wash). 2nd wash is done with 700 µl of ethanolic buffer C5, accordingly. 3rd wash is optional by repeating 2nd wash. A new MN Square-well Block (waste container) is used for 2nd and 3rd wash.

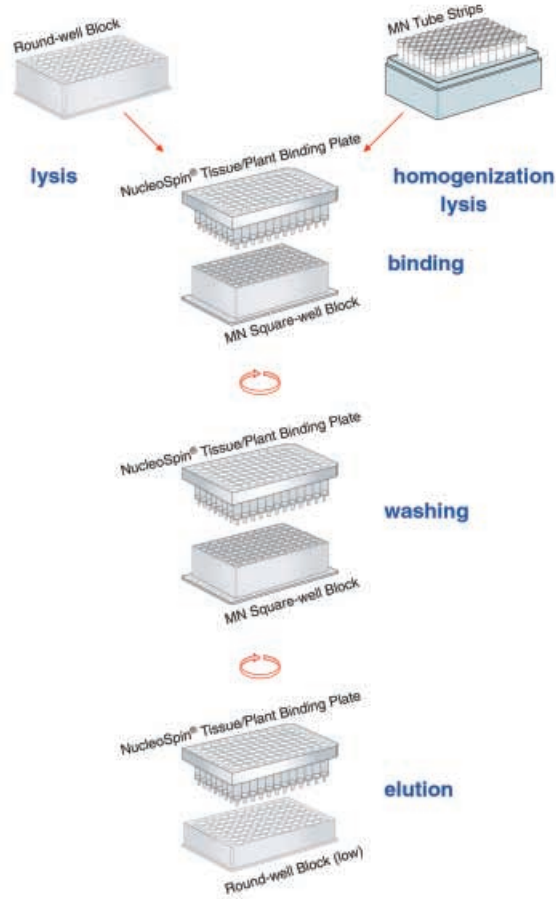


Figure 1. NucleoSpin 96 Tissue/Plant HC procedure

- 5.) *Dry:* Complete drying of the NucleoSpin Plant Binding Plate (silica membrane) is achieved by centrifugation at 5,600 x g for 10 minutes during the last wash step.
- 6.) *Elute highly pure genomic DNA:* The NucleoSpin Plant Binding Plate is stacked on a Round-well Block (low). 100 µl of buffer CE (prewarmed to 70 °C) are dispensed to each well of the plate and incubated on the membrane for 2 minutes. Genomic DNA is eluted by centrifugation at 5,600 x g for 5 minutes. Optionally, a second elution step is performed. DNA is ready-to-use for common downstream applications.

Instrumentation

The integrated automation solution consisting of the PerkinElmer MultiPROBE II HT Automated Liquid Handling System equipped with Gripper Integration Platform and integrated Robotic Centrifuge from Hettich in combination with the MACHEREY-NAGEL NucleoSpin 96-well System based on silica-membrane technology offers fully automated protocols providing high yield and high quality DNA. Instrumentation, software, plasticware, and chemistry have all been optimized for the fully automated extraction of genomic DNA from animal or plant tissue. The automation of this process replaces intensive manual protocols and therefore releases the user's time as well as removes human errors.



Figure 2. Picture of the Instrumentation, provided courtesy of J. Zimmerman (Ph.D.), EMBL.

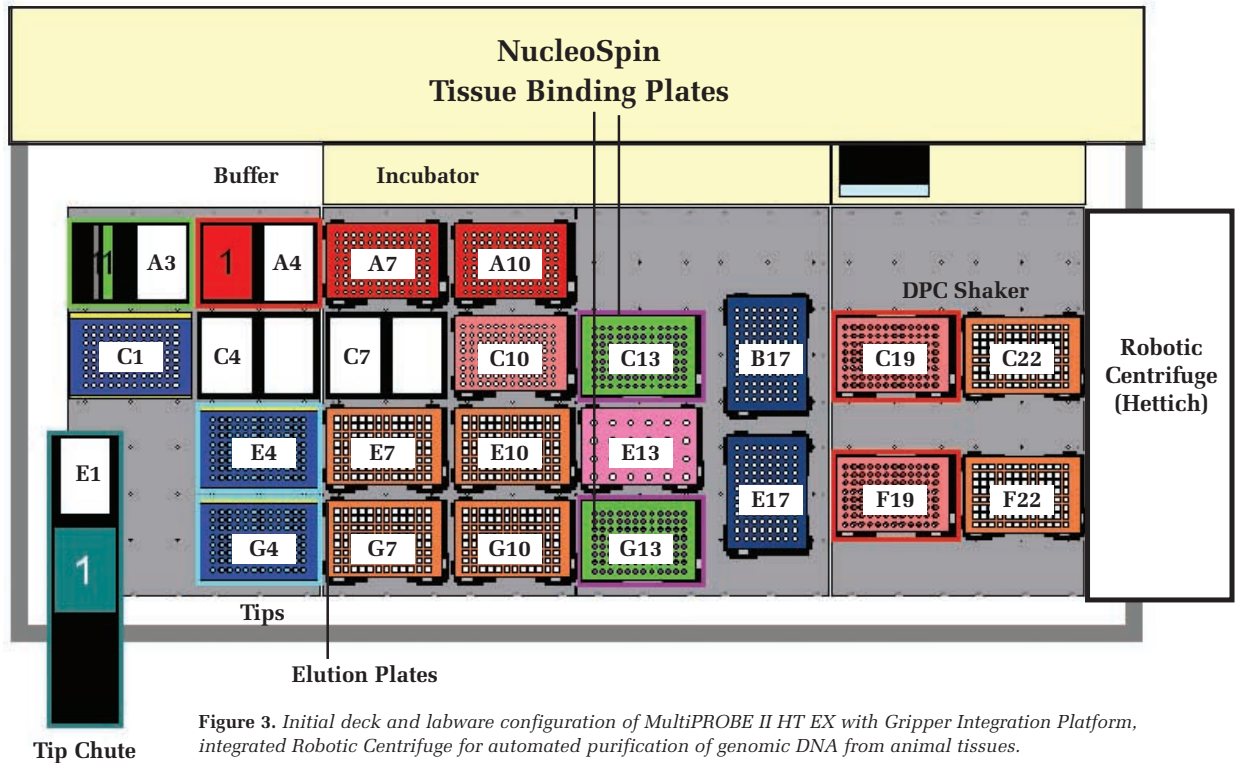
Hardware & Software Requirements

Requirements for U.S. Customers (PerkinElmer)			Requirements for International Customers Continued		
P/N	Description	Quantity	P/N	Description	Quantity
AMP8E02	MultiPROBE II HT EX with Computer	1	7001495	Computer System MPII	1
7000540	Kit, Versatip Plus HT Roborack	1	7400096	Multi COM Port BD Option Kit	1
70017091	ML Syringe Assembly, 8 Tip	1	7607537	Hanging Tip Rack Holder, MPII	2
AMPGRE0	Gripper Integration Platform	1	7601651	Microfuge Adapter 1.5 ML	1
7601716	DPC Shaker, Right Extension	1	5078889	Tile, Troughs, MPII 8 Tip	3
7001427	Left Expansion Module	1		On-Deck Peltier-Incubators	2
7400096	Multi COM Port BD Option Kit	1			
7607537	Hanging Tip Rack Holder, MPII	2			
7601651	Microfuge Adapter 1.5 ML	1			
5078889	Tile, Troughs, MPII 8 Tip	3			
	On-Deck Peltier-Incubators	2			
Requirements for International Customers (PerkinElmer)			Control Software and Instructions (PerkinElmer)		
P/N	Description	Quantity	Description	Quantity	
AMP8E01	MultiPROBE II HT EX	1	SPIN Software Install Disk	1	
7001717	Software Kit, MPII HT	1	Centrifuge Option for MultiPROBE II HT Instructions	1	
8950000	Disk Media Kit	1			
7000540	Kit, Versatip Plus HT Roborack	1			
7001709	1ML Syringe Assembly, 8 Tip	1			
AMPGRE1	Gripper Integration Platform Option	1			
7001878	8 Tip Gripper Software Kit	1			
8950000	Disk Media Kit	1			
7601716	DPC Shaker, Right Extension	1			
8950000	Disk Media Kit	1			
7001427	Left Expansion Module	1			
			Centrifuge Option for MultiPROBE II HT EX, 110 V Cat. No. 4815-31 (Hettich)		
			Description	Quantity	
			Rotanta 46 RSC Robotic, 110 V	1	
			Swing-out Rotor, 2-place	1	
			Grooved Suspension Bucket	2	
			Bucket Insert	4	
			Stand for Centrifuge	1	
			Fixation for Stand	1	
			Centrifuge Option for MultiPROBE II HT EX, 230 V Cat. No. 4815-30 (Hettich)		
			Description	Quantity	
			Rotanta 46 RSC Robotic, 230 V	1	
			Swing-out Rotor, 2-place	1	
			Grooved Suspension Bucket	2	
			Bucket Insert	4	
			Stand for Centrifuge	1	
			Fixation for Stand	1	

Labware and Chemicals

Description	Catalog Number (MACHEREY-NAGEL)	Pack Size	Key Product Features
NucleoSpin 96 Tissue HC (4 x 96 preparations)	740750.4	1 kit	Purification of genomic DNA from animal tissues (e.g., mouse tail tip sections)
NucleoSpin 96 Tissue HC (24 x 96 preparations)	740750.24	1 kit	96-well format silica-membrane technology (bind - wash - elute) HC = High Capacity *Kit includes all reagents
NucleoSpin 96 Plant HC (4 x 96 preparations)	740751.4	1 kit	Purification of genomic DNA from plant tissues
NucleoSpin 96 Plant HC (24 x 96 preparations)	740751.24	1 kit	96-well format silica-membrane technology (bind - wash - elute) HC = High Capacity *Kit includes all reagents
Lyophilized Samples (for animal tissue)	740766	1 set	1 Set of 4 vials with 800 mg of pig liver [200 mg of pig liver/vial] lysed, lyophilized sample template for demonstration of system stability and performance
Hardware Dummy Kit	740767	1 kit	Starter kit with plastic consumables for set-up and test of the automated system after installation
Additional Reagents*			
Lysis buffer T1	740760.1	1 l	Lysis buffer (for animal tissue)
Binding buffer BQ1	740761.1	1 l	Binding buffer (for animal tissue)
Lysis buffer C1	740762.1	1 l	Lysis buffer (for plant tissue)
Buffer C2 and Buffer C3	740763	800 ml 200 ml	(for plant tissue)
Proteinase K	740764	100 mg	Enzyme, lyophilized
RNase A	740765	100 mg	Enzyme, lyophilized

* Please note that kits provide all necessary reagents for running standard protocols. Additional reagents can be ordered to allow user customisation of protocols.



Deck and labware configuration of MultiPROBE II HT EX Automated Liquid Handling System with Gripper Integration Platform Workstation and integration of Robotic Centrifuge, Microplate Shaker, and Incubator. Shown is the overview of hardware and labware layout used to perform the fully automated 96-well purification

of genomic DNA from animal tissue with the NucleoSpin 96 Tissue HC kit. The deck can accommodate labware to process up to two plates without user intervention. The Robotic Centrifuge is integrated beyond the right extension of the MultiPROBE II HT EX deck.

Position Labware

A3	Wash bowl
A4	Buffer reservoirs for buffer BQ1 and buffer BE
A7, A10	Inheco incubators (empty at the beginning)
C1	1 ml Conductive tips for elution step
C4	Buffer reservoirs for buffer T1-proteinase k mix and buffer BW
C7	Buffer reservoirs for buffer B5
C10	Round-well Block for prewarming of elution buffer
C13, G13	NucleoSpin 96 Tissue HC Binding Plate on an MN Square-well Block in a centrifuge carrier
B17, E17	Centrifuge carrier for Elution Blocks (positions are empty at the beginning)

C19, F19	Round-well Blocks for samples
C22, F22	Swap positions for waste (positions are empty at the beginning)
E1	Tip Chute
E4, G4	1 ml Conductive tips for sample transfer
E7, G7	Elution Blocks
E10, G10	MN Square-well Blocks for collecting waste from wash steps
E13	Vials for RNase (optional).

Ready-to-run protocols are provided with the system. The application manuals refer to the lists of method-specific instrument and labware requirements to ensure that the robot is properly configured for automated purification. Ordering and setup will be done through your local PerkinElmer representative.

Features and Results

- **Flexibility:** genomic DNA from typically 0.5 - 1 cm (maximum: 1.2 cm) of mouse or rat tail clippings, 10 - 20 mg of animal tissue, or 50 - 100 mg of plant tissue
- **High DNA yield:** up to 40 µg of DNA from mouse tail clippings, 20 µg of DNA from animal tissue or 30 µg of DNA from plant tissue
- **High Throughput:** 96-well format. Up to 192 samples/run without manual interaction
- **High consistency of DNA yield:** typical CV values of 5-10 %
- **Fast:** 120 minutes/192 samples (without lysis)
- **High consistency of DNA quality:** typical DNA fragment size of 30-50 kbp, A260/280 ratio of 1.8 - 2.0.
- **Purification technology:** silica-membrane
- **Removal of PCR inhibitors**
- **Minimum elution volume:** 75 µl (optimum: 100-200 µl)
- **Suitability for common downstream applications:** such as restriction endonuclease digestion, Southern blotting and PCR.
- **Minimized elution dead volume**

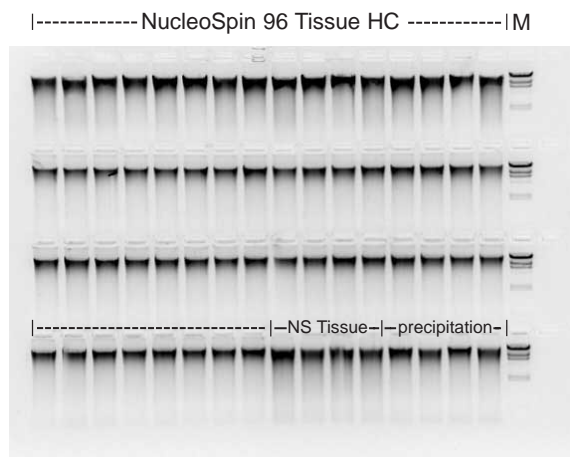


Figure 4. High consistency of DNA yield and purity from mouse tail clippings.

Comparison of genomic DNA extraction from individual mouse tails (1 clip of 0.4 cm per preparation) using different methods.

Automated extraction method using NucleoSpin 96 Tissue HC on the MultiPROBE II HT EX Automated Liquid Handling System with Integrated Robotic Centrifuge achieves 6.42 - 9.12 μg of genomic DNA. Control samples were isolated using the NucleoSpin Tissue kit for manual single preparation (NS Tissue, 8.6 - 9.5 μg) or using a precipitation method (precipitation, 10.1 - 10.7 μg). Eluted genomic DNA (10 μl /lane) was analyzed on a 0.8 % agarose gel.

M: λ /HindIII marker.

DNA migrates as a tight band with no smearing indicating the high molecular weight of about 30 kbp of genomic DNA. Low molecular weight material is not detected, indicating that RNA has been eliminated effectively. DNA yield obtained by the automated method is comparable to the DNA yield by the manual preparation and the precipitation method.

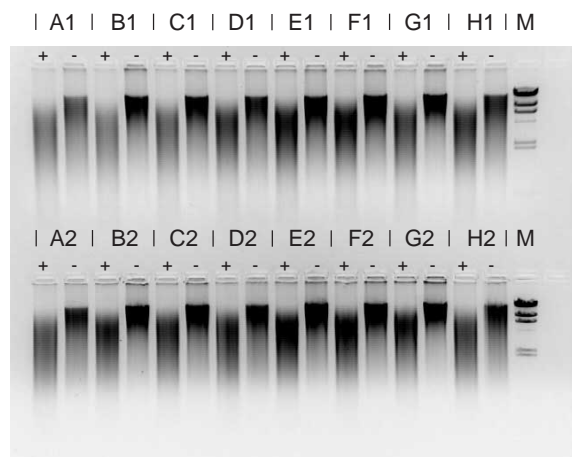


Figure 5. Genomic DNA from tissue is suitable for common downstream applications like restriction analysis.

Genomic DNA (1 μg /lane) purified with NucleoSpin 96 Tissue HC on the MultiPROBE II HT EX Automated Liquid Handling System with Integrated Robotic Centrifuge was restricted with 1 U *Eco* RI and analyzed on a 0.8% TAE agarose gel.

M: λ /HindIII marker.

+ : with digestion of DNA

- : without digestion of DNA. Samples were incubated at 37 °C in 1 x restriction enzyme reaction buffer without enzyme.

All samples containing the enzyme were restricted. No DNase activity was detected in the samples without restriction enzyme.

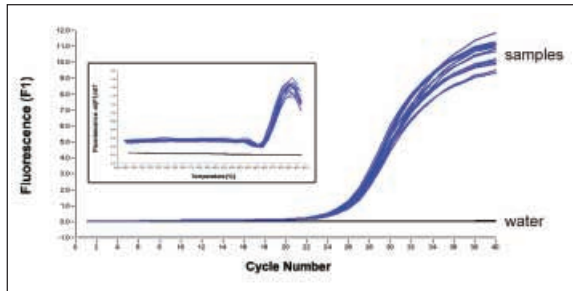


Figure 6: Processing stability analyzed by Real-time PCR.

Genomic DNA from mouse tail clippings was prepared using the automated extraction method of NucleoSpin 96 Tissue HC on the MultiPROBE II HT EX Automated Liquid Handling System with Integrated Robotic Centrifuge. 2 μ l of each 1:10 dilution of 16 randomly selected DNA eluates were used as template in a 20 μ l amplification reaction with a total of 40 cycles in a LightCycler™ analysis (SYBR® Green). The PCR product generated was 212 bp for the *Mus musculus* cytoplasmic aconitase exon (*acoI*) gene.

The figure illustrates the suitability of the extracted DNA for PCR and the high reproducibility of genomic DNA yield. A single amplification product was received (see melting curve). The obtained crossing points for all 16 samples show a mean value of 26.02 \pm 0.29 with a CV of 1.12%.

Mouse tail clipping samples and water were arrayed in adjacent wells of a 96-well plate. Genomic DNA was isolated using the NucleoSpin 96 Tissue HC on the

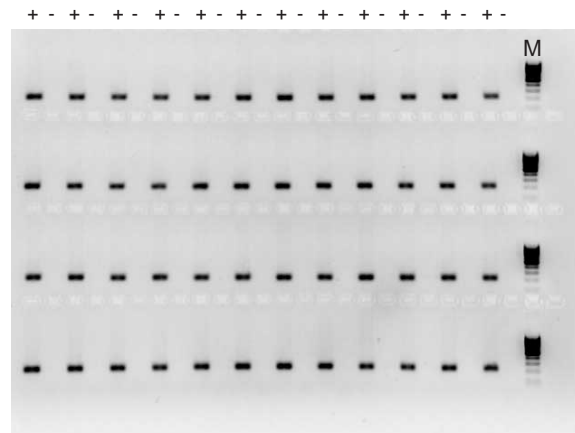


Figure 7. No cross-contamination.

MultiPROBE II HT EX Automated Liquid Handling System with Integrated Robotic Centrifuge. 2 μ l of each eluate was used for PCR with primers for the *Mus musculus* cytoplasmic aconitase exon (*acoI*) gene and running 35 cycles. PCR product from 10 μ l of each PCR assay were separated on a 1 % agarose gel. Expected PCR product from mouse tail clippings is 212 bp.

No PCR product was detected from water samples demonstrating that there is no cross-contamination.

M: [100 bp]-DNA ladder.

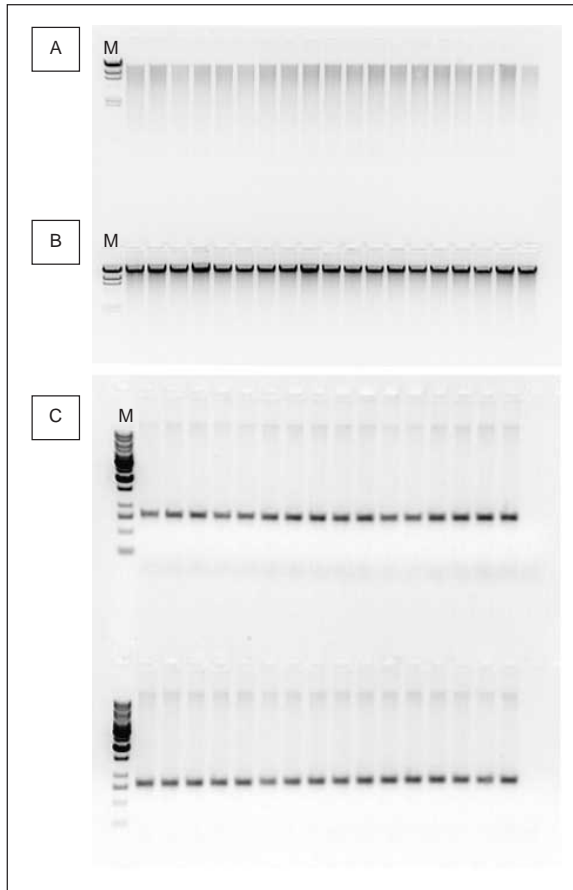


Figure 8. High quality of genomic DNA from plants.

Plant genomic DNA was extracted from wheat leaves (50 mg/preparation) using NucleoSpin 96 Plant HC. Purified genomic DNA (19 randomly selected samples, 10 μ l of eluate/lane) was analyzed on a 0.8 % agarose gel (picture 8B).

Picture B shows the homogeneity of DNA yield and quality which migrates as a tight band with no smearing indicating the high molecular weight of > 23 kbp of genomic DNA. Low molecular weight material is not detected, indicating that RNA has been eliminated effectively.

Genomic DNA purified is suitable for common downstream applications like restriction analysis (picture 8A) or PCR amplification (picture 8C).

PCR products were amplified from 2 μ l of each eluate (of a 1:10 dilution) using primers for *nad5* gene (NADH dehydrogenase, conserved mitochondrial multicopy gene) and running 35 cycles. PCR product from 10 μ l of each PCR assay was separated on an agarose gel. The expected PCR product is approximately 750 bp. Inhibitors were removed efficiently, all samples were amplified.

- A Genomic DNA, restricted with *Eco*RI (see Figure 5 for details of restriction analysis).
- B Genomic DNA, not modified
- C Genomic DNA, PCR amplified
- M marker lane.

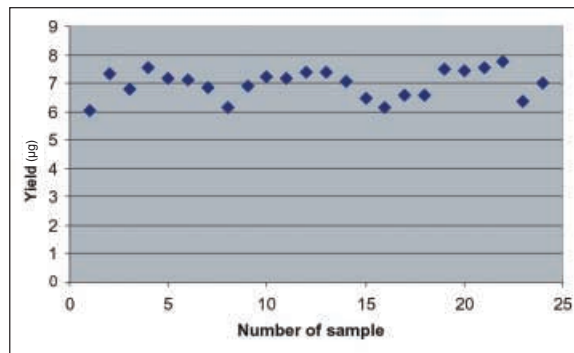


Figure 9. Reproducible yield of genomic DNA from plants

Genomic DNA was isolated from 50 mg of frozen wheat leaf tissue (n= 24) according to the standard method of NucleoSpin 96 Plant HC on the MultiPROBE II HT EX Automated Liquid Handling System with Gripper Integration Platform and integrated Robotic Centrifuge.

DNA was eluted with 150 μ l buffer CE at room temperature. Average DNA yield is 6.98 μ g +/- 0.49 μ g (mean +/- standard deviation) with a CV value of 7 %. This value proves the high reproducibility of DNA yield.

Conclusion

The automation concept of using the NucleoSpin 96-well System on the MultiPROBE II HT EX Automated Liquid Handling System with Gripper Integration Platform and integrated Robotic Centrifuge provides:

- Outstanding robustness and system stability due to the integration of multiple devices, such as a powerful centrifuge (max. speed of 6,000 x g), incubator and microplate shaker
- High degree of automation, including sample digest, for a true walk-away automation of genomic DNA purification without time consuming manual user interaction
- Processing of complex, heterogeneous and viscous samples like animal or plant tissues without risk of clogging (in contrast to vacuum technology). This is due to centrifugation which is integrated into the automated protocol. This means a minimized loss of samples
- State-of-the-art technology: high-quality DNA suitable for common downstream applications without risk of ethanol carry-over, elution dead volume as with vacuum processing, or cross-contamination
- Excellent yield of high molecular-weight genomic DNA from animal or plant tissues
- Parallel run of MultiPROBE II and centrifuge (WinPREP software with fully integrated centrifuge software)
- Flexibility of sample throughput. The system can be extended from the standard of 2 x 96 preparations per run to 4 x 96 or more by the modular deck design and the integration of appropriate storage modules.

This cooperation of PerkinElmer Life and Analytical Sciences, MACHEREY-NAGEL, European Molecular Biology Laboratory (EMBL), and Hettich means an optimized synergy of the individual expertise of the partners as worldwide supplier of instrumentation, consumables for nucleic acid purification, and as worldwide scientific reference for know-how of applications.



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