

A Semi-Automated Method for Plasmid Purification Using NucleoSpin[®] Robot-96 Plasmid on the BioRobot[®] 9600 (Qiagen)

MACHEREY-NAGEL's NucleoSpin[®] Robot-96 Plasmid Kit allows rapid isolation of high-quality plasmid DNA in the 96-well format (Fig. 1). Because of the design of the NucleoSpin[®] Plates the kit can be used on the BioRobot[®] 9600 without any modifications to the robot hardware. As the BioRobot[®] 9600 is not equipped with a gripper tool, two manual reassemblies of the vacuum chamber are required during the run. Depending on the robot configuration the time for the preparation of 96 samples is about 90 minutes.

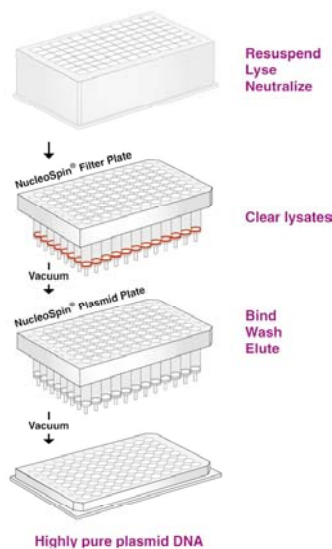


Fig. 1: NucleoSpin[®] Robot-96 Procedure

Material:

The use of the MN Frame is strongly recommended. This frame secures the right position of the NucleoSpin[®] Plasmid Binding Plate inside the robot vacuum chamber in respect to the NucleoSpin[®] Plasmid Filter Plate during the lysate filtration. Furthermore, the exact positioning of the elution plate is achieved by resting it on the MN Frame. No other parts are necessary, no changes are made to the robot configuration.

Method:

NucleoSpin[®] Robot-96 Plasmid can be adapted to the BioRobot[®] 9600 without any changes to the robot hardware. Existing scripts will not be removed nor altered.

A script can be developed based on the existing script for the QIAprep[®] 96 Turbo Miniprep Kit:

- In the Edit menu of the QIASoft[™] 3.0 open the script that is routinely used for plasmid

purifications in the 96-well format, e.g. QIAprep[®] 96 Turbo High-Speed

- Save the script under a new name, e.g. NucleoSpin[®] Robot 96 Plasmid.
- Ignore the error message and enter the new name into the protocol header. Save again.
- Change the following vacuum parameters:

Lysate filtration:

950 to 800 mbar final pressure for 3-5 minutes (pump running discontinuously). Depending on the number of cells used per well and the amount of white precipitate forming these parameters need to be adjusted with actual samples. A very smooth vacuum is recommended to prevent cross-contamination due to spraying (visible as droplets on top of the NucleoSpin[®] Plasmid Binding Plate after filtration).

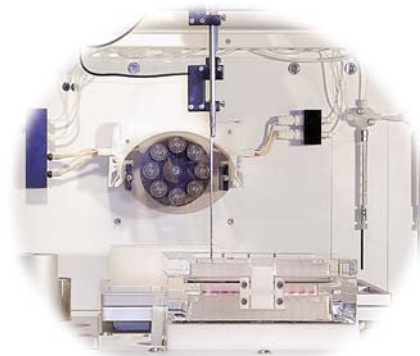
Binding:

850 to 800 mbar final pressure for 2 minutes (pump running discontinuously). The plasmid DNA should have enough time to bind to the membrane, so the flow-through rate should be at about 1-2 drops per second.

Elution:

930 to 800 mbar final pressure for 2 minutes (pump running discontinuously). It is very crucial to adjust the system to the accurate vacuum strength to prevent cross-contamination by spraying and to make sure to recover as much of the elution buffer as possible (dead volume should not be higher than about 40 µl).

Actual vacuum conditions depend on several parameters and have to be adjusted during an actual run with samples. The processing of less than 96 samples is possible by taping the unused wells of the NucleoSpin[®] Plates. At the beginning of the run the actual number of samples (or the plate positions to be processed) can be entered as usual. Also, it is possible at this stage to decide whether to use the wash buffer AW or not.



Assembly of Vacuum Chamber:

Start set-up

The MN Frame is inserted into the manifold base. The NucleoSpin® Plasmid Binding Plate is placed on top of the MN Frame. The manifold top is put into place onto the manifold base. The NucleoSpin® Plasmid Filter Plate is inserted into the manifold top.

First reassembly

After the filtration the NucleoSpin® Plasmid Filter Plate is discarded. The manifold top is removed and the NucleoSpin® Plasmid Binding Plate is placed into the manifold top. The MN Wash Plate is placed on top of the MN Frame, and the vacuum manifold is reassembled.

Second reassembly

After the washing steps the manifold is disassembled. The NucleoSpin® Plasmid Binding Plate is rested on the filter paper (supplied in the kit). The MN Wash Plate is discarded and the Elution plate (u-bottom) is placed onto the MN Frame. Alternatively, if MN Tube Strips are used for elution, the MN Frame is removed and the rack holding the MN Tube Strips is inserted into the manifold base. The manifold is reassembled and the NucleoSpin® Plasmid Binding Plate is inserted into the manifold top.

Original Data:

The NucleoSpin® Robot-96 Plasmid kit yields high-purity DNA with an average yield of 5-15 µg of plasmid DNA per 1.5 ml overnight culture (depending on starting material). The eluted DNA is ready-to use for downstream applications, like PCR, restriction analysis, automated fluorescent DNA sequencing (Fig. 2, 3).

For more information about NucleoSpin® Robot-96 Plasmid please refer to the MACHERY-NAGEL Bioanalysis Catalog or the NucleoSpin® Robot-96 Systems information bulletin.

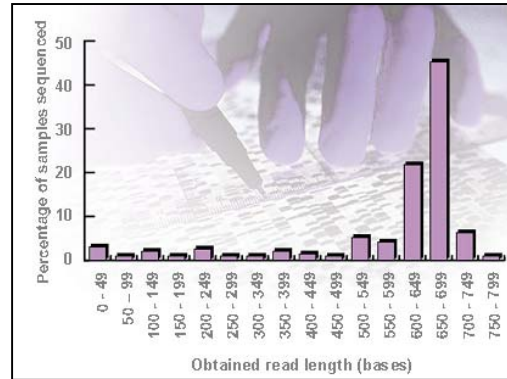


Fig. 2: Read length of customized plasmid library DNA purified from different *E. coli* strains using NucleoSpin® Robot-96 Plasmid on the BioRobot® 9600. 80% of the samples were sequenced with a read length of 550-800 bp on the ABI PRISM® 3700 DNA Analyzer.

Data kindly provided by J. Jackson, PhD, AMGEN, Dept. Of Functional Genomics, Thousand Oaks, USA.

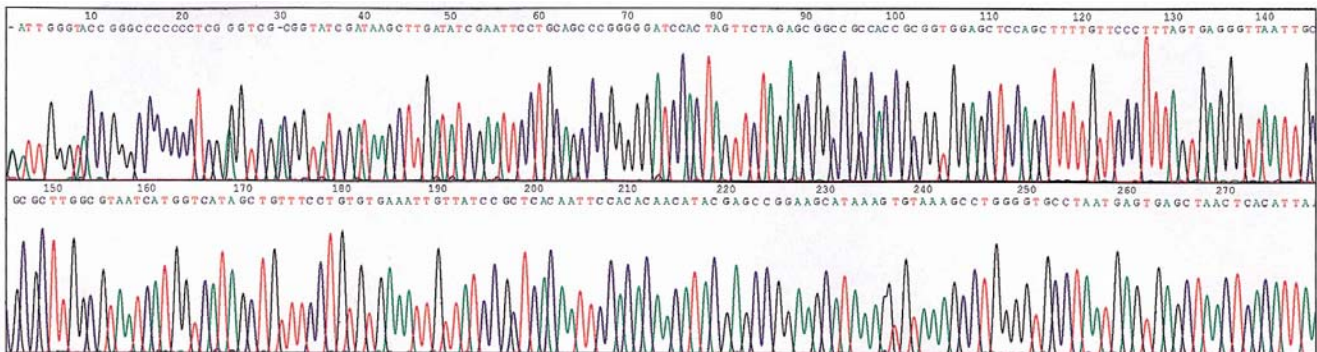


Fig. 3: BigDye™-terminator sequence of pBluescript® SK- plasmid DNA purified with NucleoSpin® Robot-96 Plasmid. DNA sequence was analyzed on an ABI PRISM® 3700 DNA Analyzer.

Ordering Information:

Product	Preps	Cat. No.
NucleoSpin® Robot-96 Plasmid	2 x 96	740 708.2
NucleoSpin® Robot-96 Plasmid	4 x 96	740 708.4
NucleoSpin® Robot-96 Plasmid	24 x 96	740 708.24
MN Frame		740 680

For more information regarding the automated use of MN products, please contact your local representative or visit MN directly under www.mn-net.com.

Trademarks: NucleoSpin is a registered trademark of MACHERY-NAGEL, BioRobot is a registered trademark of Qiagen, QIAsoft is a trademark of Qiagen, pBluescript is a registered trademark of Stratagene, ABI PRISM is a registered trademark of Applied Biosystems, BigDye is a trademark of Applied Biosystems.

MACHERY-NAGEL



MACHERY-NAGEL GmbH & Co. KG · P.O. Box 10 13 52 · D-52313 Düren · Germany · Tel. (0 24 21) 969-0 · Fax (0 24 21) 969-199
 USA: MACHERY-NAGEL Inc. · 6 South Third St., Suite 402 · Easton, PA 18042 · Tel. 610-559-9848 · Fax 610-559-9878 · Toll Free: 888-321-6224
 Switzerland: MACHERY-NAGEL AG · P.O. Box 224 · CH-4702 Oensingen · Tel. (0 62) 388 55 00 · Fax (0 62) 388 55 05
 France: MACHERY-NAGEL S.à.r.l · 1, rue Gutenberg · B.P. 135 · F-67722 Hoerd · Tel. 0388 682268 · Fax 03388 517688