

An Automated Method for Plasmid Purification Using NucleoSpin[®] Robot-96 Plasmid on the BioRobot[®] 8000 (Qiagen)

MACHEREY-NAGEL's NucleoSpin[®] Robot-96 Plasmid Kit allows rapid, fully-automated 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[®] 8000 without any modifications to the robot hardware. With the integrated gripper tool the assembly of the vacuum chamber is fully automated. Approximate processing time for the preparation of 96 samples is about 60 minutes. Up to four plates can be processed in one run.

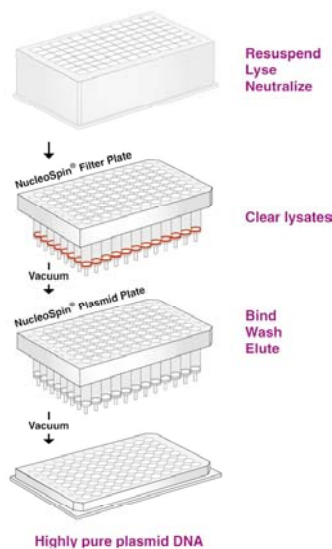


Fig. 1: NucleoSpin[®] Robot-96 Procedure

Material:

No additional equipment is required. No changes are made to the robot configuration, hardware or software.

The BioRobot[®] 8000 dries the NucleoSpin[®] Plasmid Binding Plate with vacuum and heat (up to 80°C). Neither MN Wash Plate nor MN Frame are used during the procedure.

Method:

NucleoSpin[®] Robot-96 Plasmid can be adapted to the BioRobot[®] 8000 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 Develop modus of the QIASoft[™] 4.0 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.
- In the Reagent Rotor window edit the names of the buffers, and change the names in all steps of the protocol.
- Edit the elution volume in the Variables window. NucleoSpin[®] Robot-96 Plasmid allows an elution volume between 70 µl and 150 µl.
- 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.

Drying:

800 mbar final pressure at 80°C for 3 x 3 minutes (pump running continuously).

Elution:

850 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). On some machines a vacuum of 800 mbar, pump running continuously, might be necessary to recover the DNA.

Actual vacuum conditions depend on several parameters and have to be adjusted during an actual run with samples. Settings might differ slightly from platform to platform and are also dependent on the starting material used.

Set-up:

The NucleoSpin[®] Plates are stored on the deck in the assigned positions (see deck layout info at the start of the run), with the NucleoSpin[®] Plasmid

Binding Plate on top of the NucleoSpin® Plasmid Filter Plate. Elution Plates are also placed into the appropriate positions. The MN Wash Plate is not used. Buffers are placed into the reagent rotor. All the necessary reassemblies of the vacuum chamber (binding to the membrane, drying, elution) will be performed fully automated by the integrated gripper tool. A manual interaction is not necessary.

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). The optimized outlets of the NucleoSpin® Plasmid Binding Plate and the moderate vacuum used for elution allow a processing without the risk of cross-contaminations (Fig. 3, 4), as spraying and aerosol-forming is greatly reduced.

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

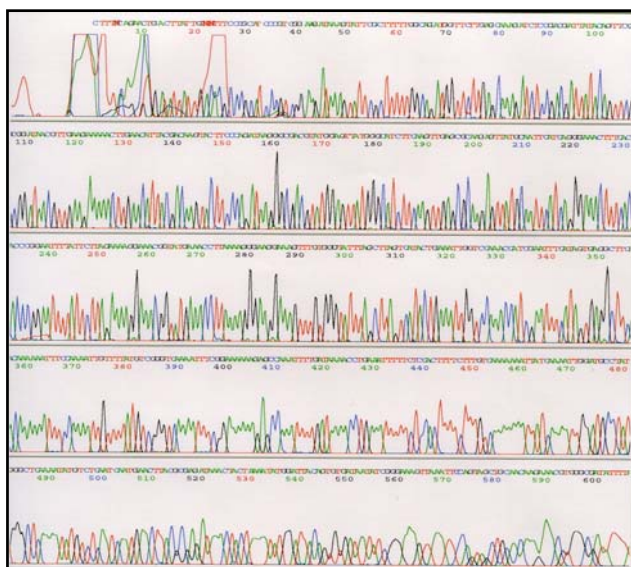


Fig. 2: Big Dye™-terminator sequence of Devgen plasmid pGN49A containing partial *C. elegans* Y44A6D.4 - gene insert taken from Devgen *C. elegans* gene library. Plasmid DNA was purified using the NucleoSpin® Robot-96 Plasmid kit on the BioRobot® 8000. Sequencing was performed on the ABI PRISM® 3100 Genetic Analyzer. Big-Dye™ used for sequencing PCR was diluted 5x. Still read lengths of up to 500 bp were obtained. All data shown was kindly provided by Wim Ornelis, Devgen N.V., Dept. Genomics, Ghent, Belgium.

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

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

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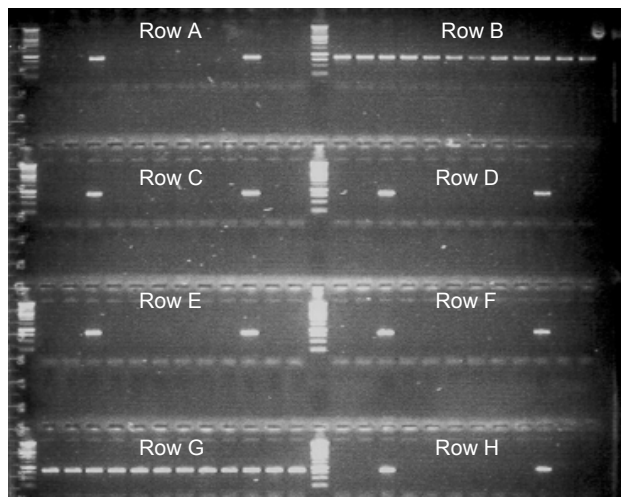


Fig. 3: PCR-results for cross-contamination check after plasmid purification using the NucleoSpin® Robot-96 Plasmid Kit on the BioRobot® 8000. A culture, containing Devgen plasmid pGN49A with *C. elegans* partial gene insert C13G3.1, was grown overnight in a deep-well plate. Only row B and G, and column 3 and 10 of the deep-well plate were filled. After purification from all 96 wells, a 20-cycle PCR reaction was performed on the elution product in order to detect possible transfer of contaminants to flanking wells during the purification process (same results for 25 cycles, data not shown). PCR-primers are flanking the gene insert (PCR-product length: 841 bp).

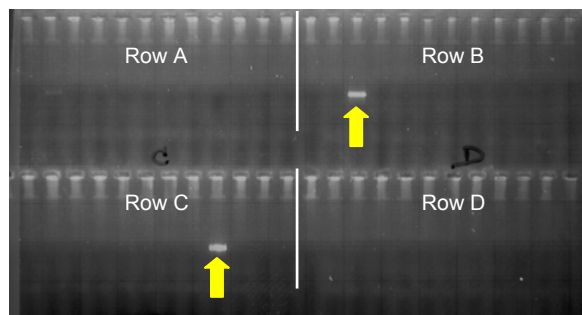


Fig. 4: Cross-contamination check after 9120 purifications (95 runs) with the NucleoSpin® Robot-96 Plasmid Kit on the BioRobot® 8000. A culture, containing Devgen plasmid pGN49A with *C. elegans* partial gene insert C13G3.1, was grown overnight in a deep-well plate. Only wells B3, F3, C9 and G9 were filled. A 25 cycle PCR was performed from all 96 wells using primers flanking the gene insert. No fragments are visible in other wells than B3, F3, C9 and G9 (data shown only for B3 and C9, arrows).