

## Application Note

### Automated Plasmid Purification with the CyBi®-RoboSpense Using MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit

Sindy Burgold-Voigt, Heidi Prüfer, Janet Kenklies, PhD, CyBio AG  
Thomas Zinn, PhD, MACHEREY-NAGEL GmbH & Co. KG, Dueren

**Keywords:** CyBi®-RoboSpense, DNA purification, plasmid DNA, NucleoSpin® Robot-96 Plasmid Kit

**Abstract:** The MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid kit technology for purification of plasmid DNA from bacterial cultures has been automated on the CyBi®-RoboSpense. The vacuum filtration accessories, robotic microplate handler and shaker for microplates allowed performing a fully automated extraction procedure. The high quality of extracted DNA was shown by PCR and restriction digestions. Purified plasmid DNA provided high quality sequence data (reading length >800nt, 99.9 % accuracy). Average yield of plasmid DNA was 9µg from 1.5mL bacterial cultures.

**Introduction:** The NucleoSpin® Robot-96 Plasmid procedure uses a modified alkaline lysis plasmid miniprep protocol. Bacterial cultures are harvested by centrifugation. After resuspension of the pelleted bacteria and alkaline lysis a neutralization and binding buffer containing chaotropic salt is added. The crude lysates are cleared by vacuum filtration with the NucleoSpin® Plasmid Filter Plate directly into the NucleoSpin® Plasmid Binding Plate.

The plasmid DNA binds reversibly to the silica membrane in the Binding Plate during a second vacuum driven filtration step. After subsequent washing and drying steps, highly pure plasmid DNA is eluted with elution buffer or water. This protocol was automated using the CyBi®-RoboSpense.

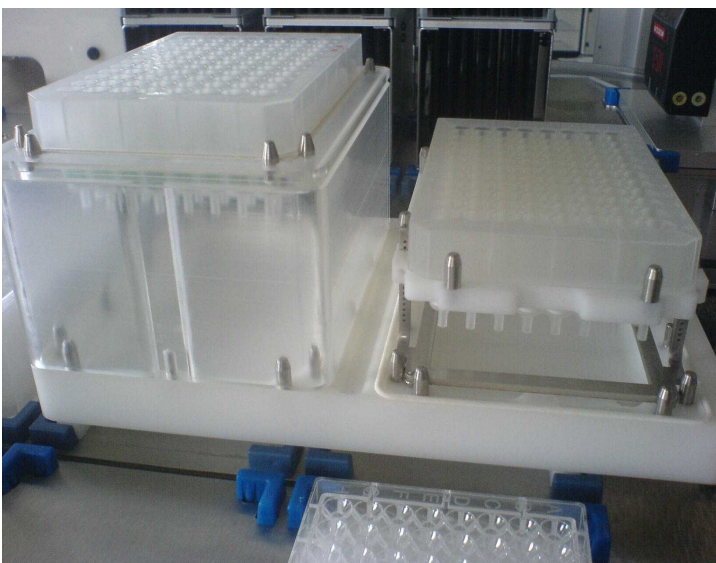


Figure 1: CyBio's Vacuum Chamber for processing NucleoSpin® Robot-96 Plasmid Kit

## Application Note

**Methods:** The MACHERY-NAGEL NucleoSpin® Robot-96 Plasmid kit (2x96 preps, MN Cat. No. 740708.2) extraction protocol was set up on a CyBi®-RoboSpense 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 1–1000µL, a vacuum manifold, a shaker for microplates and a robotic microplate handler.

The purification procedure was established according to MACHERY-NAGEL's standard protocol for NucleoSpin® Robot-96 Plasmid kit. The protocol was carried out with 1.5mL bacterial suspension (*Escherichia coli* DH5α) per well. The quality and integrity of the purified DNA 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  $A_{260/280}$  ratio with a SpectraMax 250 microplate reader. For additional quality checks, PCR amplification of a gene fragment of plasmid pGEM®-T (750bp) was performed with 35 PCR cycles. Enzymatic restriction of plasmid pGEM®-T by *Nco*I and *Not*I produced two fragments. Purified plasmid DNA was analyzed by MWG sequencing service on the ABI 3730 XL DNA Analyzer.

### Automated extraction procedure:

1. Bacterial cells were cultivated at 37°C, 120rpm in a flask and harvested after 18 hours ( $OD_{600}=2.7$ ) by centrifugation at 1,000xg for 10min. The plate was placed on the CyBi®-RoboSpense deck and the automated method started. All subsequent steps were performed by the robot.
2. Supernatant was discarded. 250µL Resuspension Buffer A1 was added to wells containing bacteria pellets, with pipetting 3 times up and down and shaking for 6min at 1000rpm to resuspend cells.
3. 250µL Lysis Buffer A2 was added, with mixing by shaking at 500rpm for 5min.
4. 350µL Neutralization Buffer A3 was added, with gentle mixing by pipetting up and down three times (pipetting speed 100µL/sec), and while shaking for 1min at 750rpm.
5. The crude lysate was transferred to the NucleoSpin® Plasmid Filter Plate followed by a 2min wait.
6. Lysate was cleared with vacuum filtration directly into the NucleoSpin® Plasmid Binding Plate at 800mbar for 3min.
7. The vacuum manifold was reassembled with the robotic microplate handler, and DNA bound to the NucleoSpin® Plasmid Binding plate during vacuum filtration for 1min at 900mbar.
8. The silica membrane was washed three times by adding 600µL Wash Buffer AW and 2x 900µL Wash Buffer A4, followed by application of vacuum for 1min at 600mbar.
9. The membrane was dried using the automated column drying mode with vacuum for minimum 12min.
10. Purified DNA was eluted by adding Elution Buffer AE, 75µL for the first elution step, and 150µL for a second elution step. After a 1min wait, vacuum was applied for 1min at 600mbar for both steps.

**Results:** Purified plasmid DNA was of high quality, as analyzed by gel electrophoresis (Figure 2) and was successfully used for a downstream PCR amplification (Figure 3) and restriction analysis (Figure 4). Average DNA yield was 9µg from 1.5mL bacterial cultures. Purity as determined by an OD 260nm/280nm ratio of 1.7, was excellent.

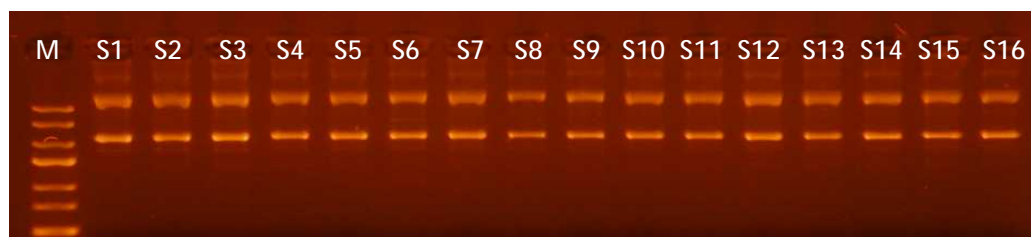
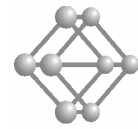


Figure 2: Analysis of extracted plasmid DNA by agarose gel electrophoresis. Lane M: 6µL GeneRuler™ Express DNA ladder (Fermentas), lanes S1-S16: 2.5µL sample.



## Application Note

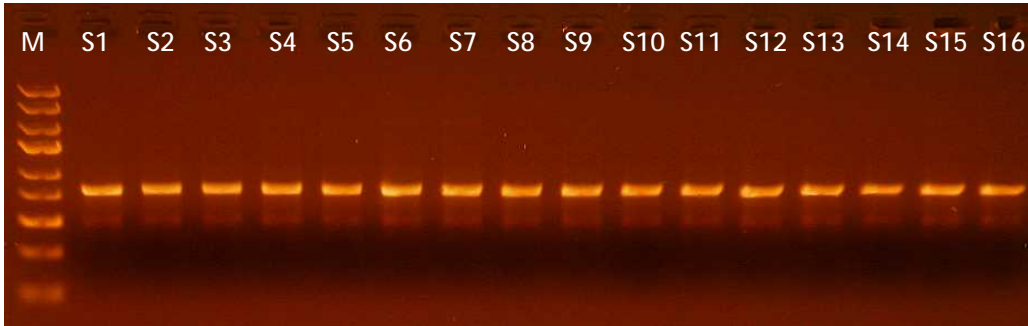


Figure 3: PCR amplification of purified plasmid DNA. A 750bp pGEM<sup>®</sup>-T gene fragment was amplified by PCR with primer T7 and SP6 with 35 cycles and analyzed by gel electrophoresis. Lane M: 6µL GeneRuler<sup>™</sup> Express DNA Ladder (Fermentas); lanes S1-S16 : 5µL PCR product.

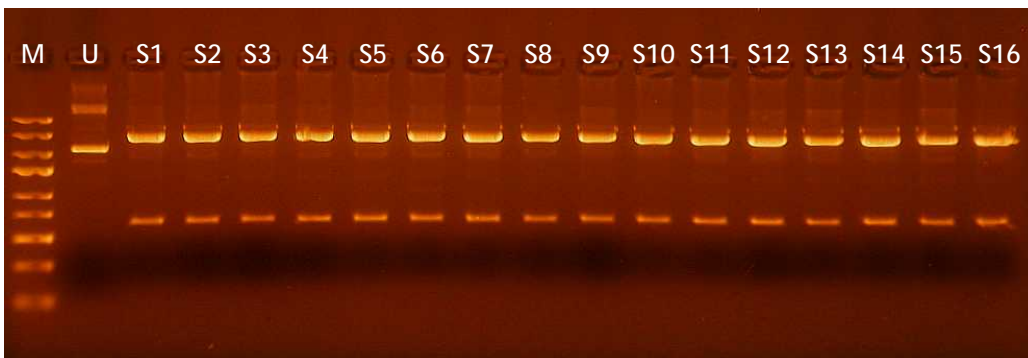


Figure 4: Restriction digestion of plasmid pGEM<sup>®</sup>-T by Nco I and Not I (Jena Bioscience GmbH). First lane: 6µL GeneRuler<sup>™</sup> Express DNA Ladder (Fermentas), lane U: 2.5µL undigested DNA sample, lanes S1-S16: 10µL digested sample.

Sequencing was performed on the ABI 3730 XL DNA Analyzer. Purified sequencing reactions delivered long sequence reads (>800nt) with high quality, as demonstrated by electropherogram (Figure 5 and 6).

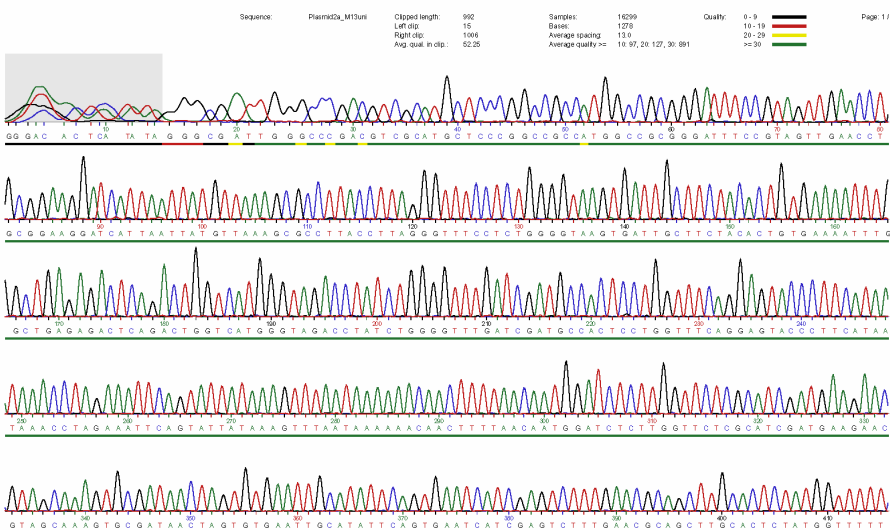


Figure 5: First part of the electropherogram of a partial sequence of plasmid with a reading length of about 800 nucleotides.

# Application Note

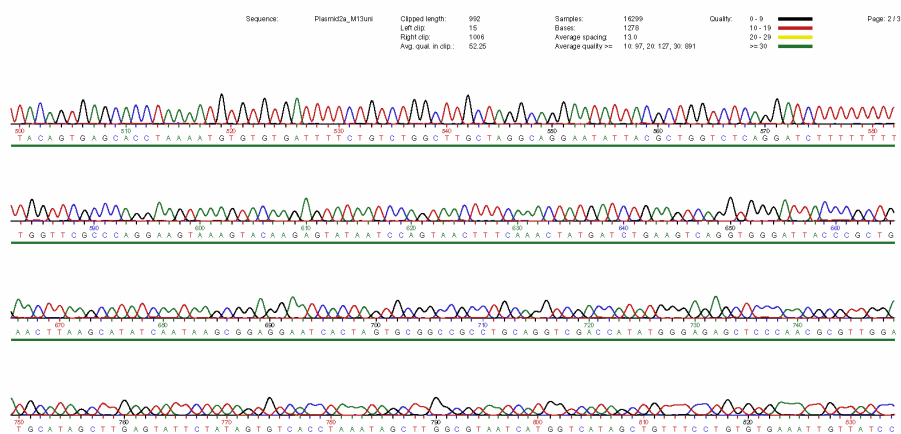


Figure 6: Second part of electropherogram of a partial sequence of plasmid. An average sequence length of 600 bp with an accuracy of >99% was obtained.

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

Several adjustments to the method optimized the automated procedure. To thoroughly resuspend the bacterial pellets and to increase the DNA yield, the shaking time for the resuspension step was increased to 6min after pipetting up and down three times.

It was also important to clear the lysates completely. Therefore, lysis was completed under continuous, gentle shaking for 5min at 500rpm. By pipetting the suspensions 3 times and by shaking for 1min at 750rpm after addition of Neutralization Buffer A3, optimal formation of precipitates was achieved. Careful adjustment of pipetting speeds, implementation of post-aspiration and -dispensing delays (2000ms), and use of air gaps ensured reliable transfer of the viscous crude lysates to the NucleoSpin® Filter Module.

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

The CyBio® EluteControl software vacuum evaluation mode enabled the user to toggle between execute and edit modes during a run. Thus, for example lysate elution 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 in 75µL and in a second step with 150µL elution buffer at 600mbar to assure full elution of DNA and to increase yields.

**Conclusion:** The results demonstrate that the CyBi®-RoboSpense is very well suited for reliable automation of vacuum-based plasmid DNA extraction from bacterial cells using MACHEREY-NAGEL's NucleoSpin® Robot-96 Plasmid kit technology, using its specialized accessories for vacuum filtration and shaking, as well as optimization of pipetting parameters and vacuum settings.