

Application Note

Automation of Genomic DNA Purification from Blood with the CyBi®-RoboSense Using the MACHERY-NAGEL NucleoSpin® 8/96 Blood Kit

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Abstract: The MACHERY-NAGEL NucleoSpin® 8/96 Blood kits for purification of genomic DNA from blood were automated on the CyBi®-RoboSense. The extraction procedure was completely automated using the vacuum device, robotic microplate handler, the temperature controlled unit and shaker for microplates. Quality of purified DNA was shown by agarose electrophoresis and PCR. Genomic DNA was extracted with very good yields (5 µg from 200 µL fresh blood) and excellent purity (A 260 nm/280 nm = 1.9).

Introduction: NucleoSpin 8/96 Blood kits are designed for the parallel isolation of genomic DNA from whole blood. After lysis of blood cells genomic DNA is selectively bound to a silica membrane while contaminants are washed away effectively. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

Due to its plate design and the possibility to perform the complete procedure under vacuum NucleoSpin® 8/96 Blood can be easily adapted to The CyBi®-RoboSense for fully automated processing without manual interactions. In this application note, we demonstrate the fully automated NucleoSpin® Blood extraction method for genomic DNA from blood on the CyBi®-RoboSense.

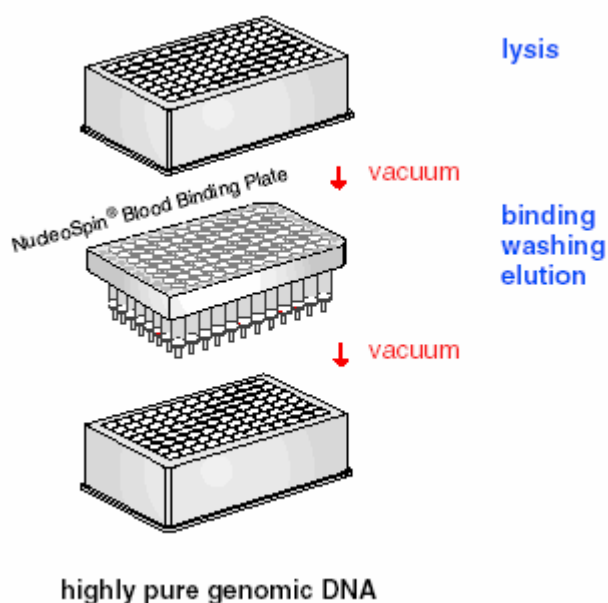


Figure 1: General procedure of the NucleoSpin® 96 Blood Kit

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Methods: The MACHEREY-NAGEL NucleoSpin® 8 Blood kit (12x8 preps, MN Cat. No. 740664) or NucleoSpin® 96 Blood kit (1x96 preps, MN Cat. No. 740665.1) extraction protocols were 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 1–1000 µL, a vacuum manifold, a temperature controlled board, a shaker for microplates and a robotic microplate handler. Using the NucleoSpin® 8 Blood kit multiples of eight up to a maximum number of six 8-well strips for a total of 48 samples can be processed simultaneously whereas NucleoSpin® 96 Blood allows for purification of up to 96 samples per run. 200 µL of fresh blood were used for verification of the automated method. The quality and integrity of the DNA obtained was analysed by gel electrophoresis and ethidium bromide staining. Yields and purity of extracted DNA were determined by measurement of the absorbance at 260nm and 280nm with a Lambda Scan 200 microplate scanning spectrophotometer (MWG Biotech) and by calculation of the $A_{260nm/280nm}$ ratio. For additional quality check, PCR amplification of a β -actin fragment (200bp) was performed on iCycler (BIORAD) with 35 PCR cycles.

Automated extraction procedure:

1. 200 µL blood samples were transferred into the wells of a Lysis Block. The plate was placed on the CyBi®-RoboSpense deck and the automated method started.
100 µL of a premix of buffer BQ1 and proteinase K (75 µL buffer BQ1, 25 µL proteinase K) was added to each sample, mixed by pipetting up and down for 3 times and by shaking for 10 min at 1200 rpm.
2. 400 µL of a premix of ethanol and buffer BQ1 (200 µL ethanol, 200 µL buffer BQ1) were added and mixed by pipetting up and down 3 times and by shaking for 0.5 min at 1000 rpm.
3. The mixture was transferred to the NucleoSpin® Blood Binding Strips/Plate.
4. Lysate was overlaid with 150 µL buffer B5.
5. Genomic DNA was bound to the silica membrane with vacuum filtration, 800 mbar for 5 min.
6. The silica membrane was washed three times by adding 600 µL of wash buffer BW and 2 x 900 µL wash buffer B5. For each wash step, vacuum was applied for 5 min at 800 mbar.
7. The MN Wash Plate was removed from the vacuum manifold with the robotic microplate handler disassembling and reassembling the vacuum manifold. The membrane was dried using the automated column drying software mode with full vacuum for a minimum of 10 min.
8. Purified DNA was finally eluted by adding 100 µL of pre-warmed Elution Buffer BE. Elution step was repeated once. Vacuum was applied for 1 min at 600 mbar for both steps.

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Results: To analyze the quality of the purified DNA, randomly selected samples were subjected to agarose gel electrophoresis and tested in PCR. Yield as calculated from A_{260nm} reads and purity as determined by an $A_{260nm/280nm}$ of 1.9, were excellent.

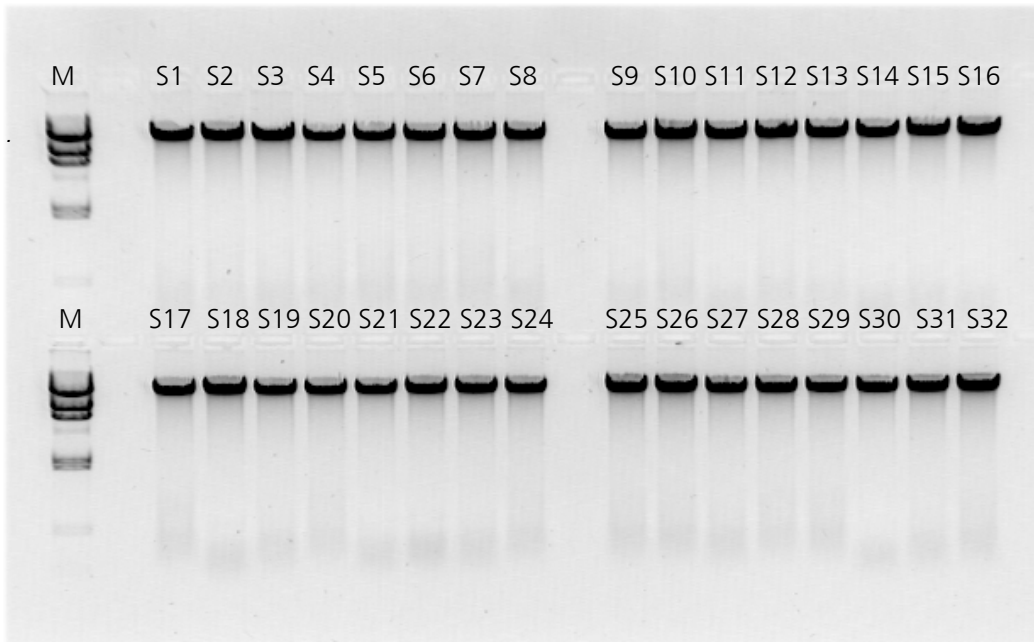


Figure 2: Agarose gel analysis of 32 samples of purified genomic DNA from blood. Lane M: lambda Hind III size standard, 6 μ L (Fermentas), lanes S1-S32: 15 μ L sample from fresh blood. No samples were loaded on lanes adjacent to S8 and S24. Genomic DNA from all samples was prepared from 200 μ L whole blood using NucleoSpin® 96 Blood on the CyBi®-RoboSpense fully automated under vacuum. High reproducible yields were obtained. The presence of a high molecular weight migrating band of genomic DNA and the absence of low-molecular weight smear underline the quality of purified DNA

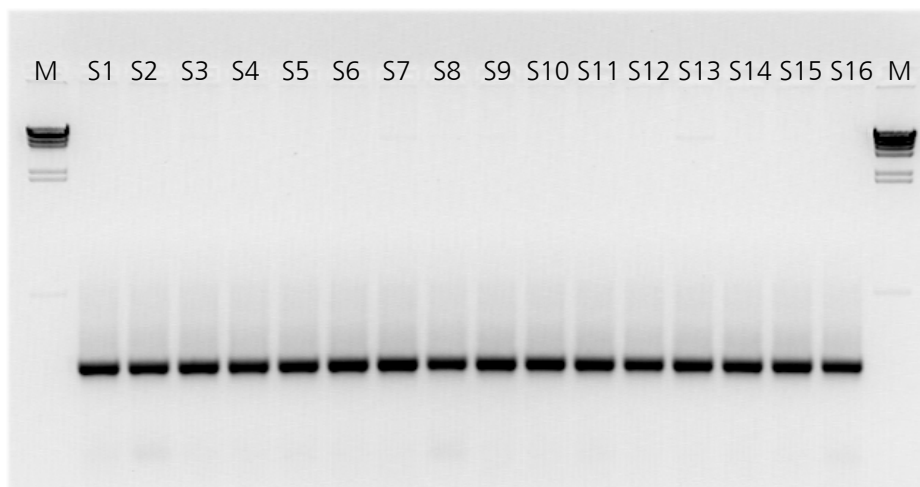


Figure 3: PCR amplification of purified genomic DNA. A 200bp β -actin fragment was amplified by PCR with 35 cycles. Lane M: lambda Hind III size standard, 6 μ L (Fermentas), lanes S1-S16: 5 μ L PCR product. All samples were amplified with high reproducibility. No inhibition was observed.

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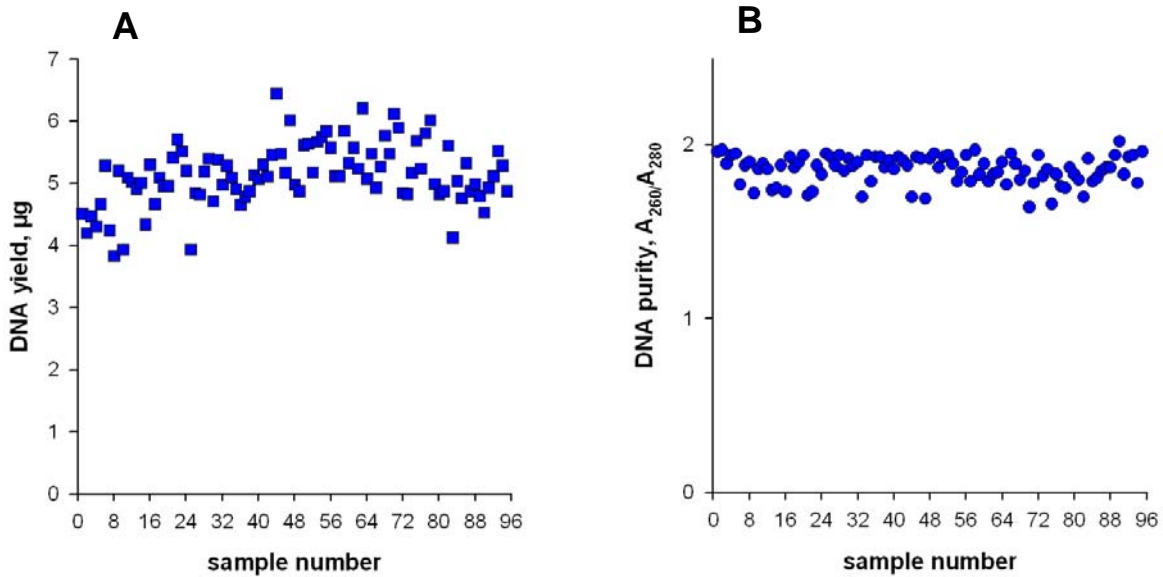


Figure 4: 96 aliquots from the same blood sample have been purified fully automated on a CyBi®-RoboSpense using the NucleoSpin®96 Blood kit. The average yield is 5.13 μg DNA (A) and the average purity is A_{260}/A_{280} 1.86 (B).

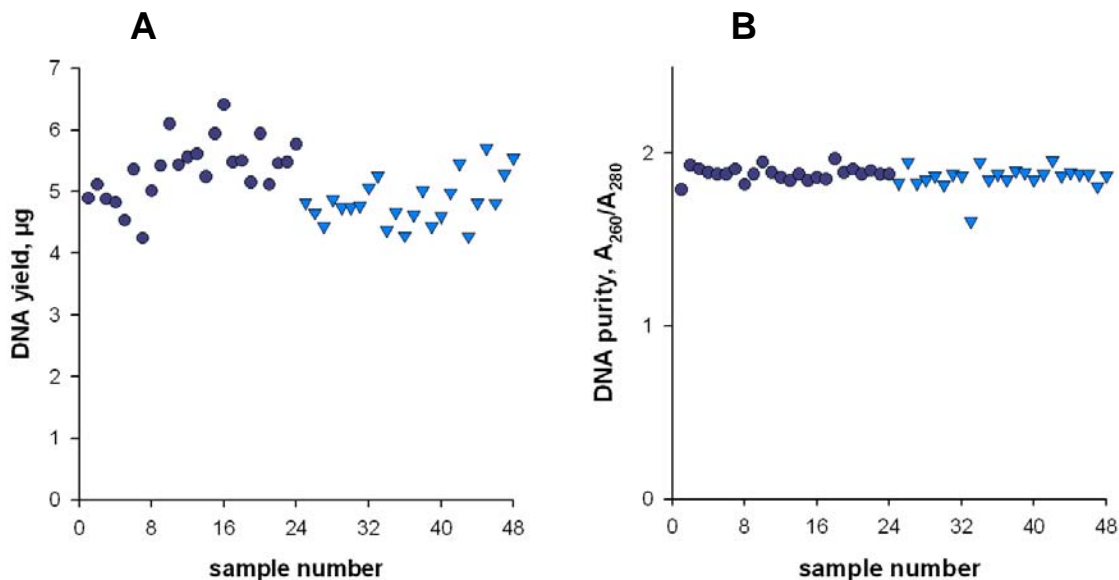


Figure 5: DNA isolation from fresh and frozen individual blood samples. 24 aliquots from fresh blood samples (circles) and 24 aliquots of frozen samples were purified fully automated on a CyBi®-RoboSpense using the NucleoSpin® 8 Blood kit. DNA yield (A) and purity A_{260}/A_{280} were determined photometrically (B). High consistent yields (average 5.38 μg for fresh samples, 4.85 μg for frozen samples) and purities (average 1.88 for fresh samples and 1.86 for frozen samples) were obtained.

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Discussion: Genomic DNA was isolated from 200 μL fresh and frozen blood samples. DNA with high structural integrity was obtained from each sample as indicated by the absence of low molecular weight smear on agarose gel. Excellent purity was determined by an $A_{260\text{nm}}/A_{280\text{nm}}$ ratio of 1.9 and by PCR amplification.

The use of NucleoSpin Blood Binding Strips allowed processing of NucleoSpin 8-well strips stacked on 96-well blocks and thus adapt reagents and consumables to the user specific number of samples. 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, allowing exquisite control over the automated vacuum process. 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 with 100 μL at 600 mbar to assure full elution of DNA and to increase yields.

Conclusion: The results demonstrate that the CyBi®-RoboSpense is very well suited for the reliable automation of vacuum-based genomic DNA extraction from blood using MACHERY-NAGEL's NucleoSpin® 8/96 Blood kit technology. The combination of the CyBi®-RoboSpense liquid handling instrument with its specialized accessories for vacuum filtration, on-deck shaking and heating, and the robust and reliable NucleoSpin® Blood kit chemistry allowed for reliable extraction of nucleic acids with a minimum of hands on time for the user.