

Application Note

CyBio CyBi[®]-Genomics Workstation/ Macherey Nagel Nucleofast[®] 96 PCR Automated PCR product purification with CyBi[®]-Genomics Workstation using Nucleofast[®] 96 PCR Plates

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The CyBi[®]-Genomics Workstation has been used to automate Macherey Nagel NucleoFast[®] 96 PCR technology for ultrafiltration based PCR product purification. The use of a 96-well pipetting head with free horizontal positioning and vacuum processing not only enables a rapid (17 min) automated procedure, but also maintains the purification technology's highly reliable performance. The result is excellent yields (83 %) of highly purified DNA ($A_{260/280} > 1.8$).

Introduction: This purification method is based on the removal of contaminants such as dNTPs, salt and primers from the desired PCR product by ultrafiltration through a membrane. The automated purification protocol consists of one vacuum-processed filtration step, followed by resuspension of the purified dry PCR product and subsequent transfer into an elution plate. An optional washing step can be included prior to resuspension.

Methods: The protocol was set up on a 96-well automated pipettor with a volume range of 1 – 250 μ l, mounted on a 10 position deck, equipped with an electronically controlled vacuum pump, a vacuum manifold for filter plates, the patented SafeElute sealing system and a stationary plate gripper (all CyBio). Purification procedure was established according to the Macherey Nagel standard protocol for NucleoFast[®] 96 PCR Plates. The whole protocol was carried out with 20 μ l PCR product of 800 bp length, corresponding to 10 μ g DNA per well. Quality and length of DNA was analysed by gel electrophoresis (1 % agarose in TBE buffer; marker: 4 μ l Low DNA Mass Ladder, Invitrogen) and ethidium bromide staining. DNA purity was determined by absorbance ratio at 260nm/280nm (UV spectrophotometer: Spectra Max, Molecular Devices; quartz micro plate: Hellma). Recovery rate was calculated after DNA quantification with SYBR Green I (Roche Applied Science) by mixing 5 μ l sample with 95 μ l TE buffer and 100 μ l 2 x SYBR Green I (5000-fold dilution), followed by 10 min dark incubation and subsequent fluorescence analysis at 485nm/535nm ($\lambda_{ex}/\lambda_{em}$, detector: Victor 1420 Multilabel counter, Wallac; plates: Greiner Bio-one, # 655076) using a DNA calibration curve.

Automated procedure:

1. Add TE buffer to unpurified PCR product in a thermocycler plate to a final volume of 100 μ l, mix and transfer sample to a NucleoFast[®] 96 PCR plate placed on a vacuum manifold
2. Tightly seal NucleoFast[®] 96 plate to vacuum manifold using SafeElute and apply vacuum at a pressure difference of 600 mbar to ambient for 10 minutes, wash tips in parallel
3. For an optional wash step, add 100 μ l TE buffer and repeat the vacuum step (step 2). The DNA sample is retained in the NucleoFast[®] 96 plate.
4. Move NucleoFast[®] 96 plate from vacuum manifold to empty deck position
5. Dissolve purified PCR product by adding 50 – 100 μ l TE buffer to dry PCR product and by mixing 30 times just above the membrane surface using the 96-well parallel pipetting head
6. Collect dissolved PCR product from NucleoFast[®] 96 plate by aspirating from five different positions on the ultrafiltration membrane using the free horizontal positioning mode and dispense sample into an elution plate.

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Results: Purified DNA was of high quality, as analysed by gel electrophoresis (figure 1) and high purity, as determined by absorbance ratio at 260/280 nm ($A_{260/280}$ $\Delta\Delta$ 1.8). Recovery, as determined by SYBR Green I quantification, was 83 % (n = 90, cv = 17). The volume remaining in the filter plate after aspiration of resuspended DNA was only 2.7 μ l per well ('dead volume'). Automated sample processing took 17 minutes without - and 27 minutes with – the optional washing step. A summary of these results, in comparison to Macherey Nagels specifications for manual execution, is listed in table 1.

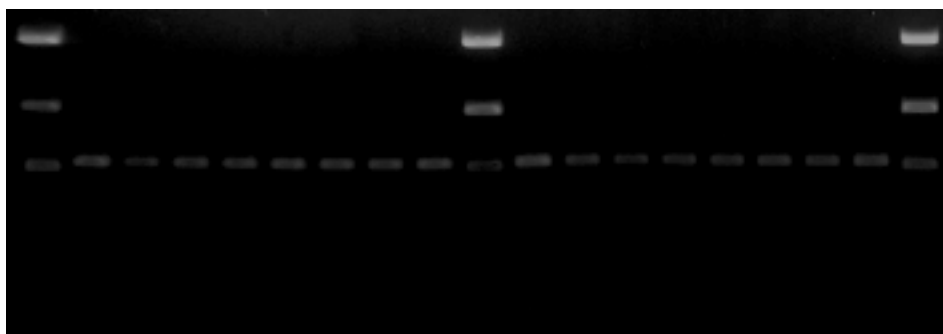


Figure 1:
Analysis of unpurified and purified PCR products by gel electrophoresis: Samples containing 20 μ l (10 μ g) of an 800 bp PCR product were purified by two 10 minutes filtration steps (including the optional washing step) and recovered in 50 μ l TE buffer. Equivalent amounts of corresponding PCR products before and after automated purification were analyzed.

Table 1:
Technical specifications obtained by automated PCR product purification in comparison to manual PCR product purification (Macherey Nagel NucleoFast 96 PCR user manual) .

Specification	CyBi®-Genomics Workstation	manually
Dead volume	2.7 μ l	3 – 4 μ l
Purity	$A_{260/280}$ $\Delta\Delta$ 1.8	$A_{260/280}$ $\Delta\Delta$ 1.7 – 1.8
DNA recovery	83 % for DNA fragments of 800 bp (n = 90, cv = 17)	50 – 95 % for DNA fragments > 150 bp
Time:	17 min (27 min w/ optional washing step)	20 min

Discussion: By applying special features of the robotic system to the purification method, it was possible to establish a very safe and fast process with excellent specifications very good comparable to those published by Macherey Nagel (table 1). Processing 96 wells in parallel enabled faster execution of mixing and resuspension steps, especially in step 5 for DNA recovery. Preparation time was decreased further through use of a software command for parallel execution of vacuum steps and tip washing. Careful adjustment of piston speeds was implemented to ensure aerosol free pipetting and to prevent liquid splashing. This prohibited the formation of liquid droplets on the well wall that may have otherwise escaped vacuum filtration and interfered with DNA purity and recovery. The free horizontal positioning of pipette tips in the plates proved to be very beneficial for aspiration of the purified and resuspended PCR product from the ultrafiltration membrane, resulting in very small dead volume, and consequently maximal DNA recovery rates. The results obtained demonstrate clearly that the CyBi®-Genomics Workstation can be used for fast and efficient automation of PCR product purification with Macherey Nagel NucleoFast® 96 PCR technology.