Genomic STARlet: High throughput PCR purification using ultrafiltration technology

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Properly purified PCR products are crucial for many molecular biological applications. When a large number of different products are required the purification procedure has to deal with many samples in parallel. Moreover, it needs to be fast and reliable.

- Increase speed for cleaning up PCR reactions
- Facilitate high throughput in order to create multiple purified PCR products in one run
- Ensure reproducibility of the clean-up procedure

Introduction

PCR (polymerase chain reaction) is an indispensable tool in most areas of Life Sciences. Resulting PCR products are used for the generation of DNA sequence information of genes and their variations, for synthesis of highly specific DNA probes, for generating templates for the production of biologicals, and for qualitative as well as quantitative analysis of DNA and RNA materials.

When using the PCR clean-up products, removal of components such as dNTPs (deoxynucleoside triphosphates), primers and salts is essential for many downstream applications in order to avoid their interference with these reactions. However, PCR product purification is often a tedious manual multistep procedure, in which DNA needs to bind and washed several times. This has prevented frequent use of PCR products in many high throughput applications.

Hamilton and MACHEREY-NAGEL have developed a fully automated PCR purification method using the NucleoFast® PCR clean-up technology from MACHEREY-NAGEL on the Genomic STARlet liquid handling workstation (Figure 1).

The process is based on removal of contaminants by ultrafiltration in a 96-well format. There is just one sample loading step and an optional quick washing step. There is no need for binding and elution steps which makes the procedure extremely fast, efficient and easy to automate by using a vacuum station for liquid removal.

Figure 1: Genomic STARlet

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Reliable high throughput PCR purification achieved by Hamilton's fully automated liquid handling and filtration system

With the Genomic STARlet, Hamilton offers a fully automated solution for purifying PCR products using MACHEREY-NAGEL's NucleoFast® PCR kits.

Kit Description

NucleoFast® PCR kits are based on ultrafiltration and they are designed for rapid clean-up of PCR fragments in a high throughput format. The PCR samples are applied onto the ultrafiltration membrane at the bottom of each well in the NucleoFast® 96 PCR plates (see Figure 2). Molecules such as primers, dNTPs and salts which might interfere with or even block downstream applications are filtered to waste by using vacuum. PCR products larger than 150bp in length are retained on the membrane and can be recovered from the membrane by adding water or low salt buffer after a short incubation. The purified PCR fragments can be directly used in downstream applications. The NucleoFast® procedure eliminates the use of chaotropic salts for binding of nucleic acids and subsequent ethanolic washing steps.

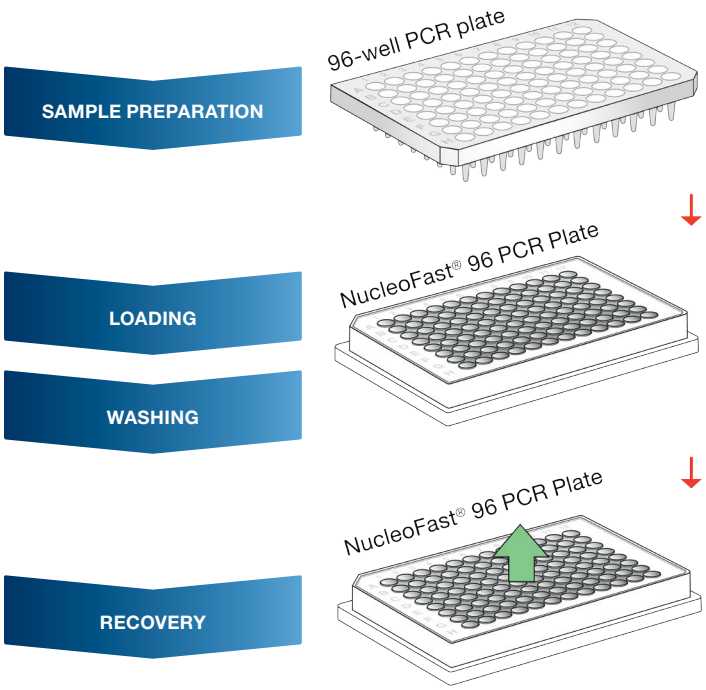

Figure 2: Workflow of the PCR product purification method. Samples are transferred from a 96-well PCR plate to a NucleoFast® 96 PCR Plate (loading). After an optional washing step (washing) they are recovered from the ultrafiltration membrane by adding RB buffer (recovery).

Method Description

The PCR plates are manually placed onto the source position of the Genomic STARlet. User dialog driven, the PCR reaction is adjusted to 100µl volume with sterile water and transferred to the NucleoFast® 96 PCR Plate. PCR products are collected on the surface of the ultrafiltration membrane, while contaminants are removed by applying vacuum. A wash step with 100µl sterile water is optional. 50–150µl of recovery buffer (RB) or sterile water is added to the purified PCR product and after a short incubation period the PCR product is aspirated from the NucleoFast® Plate and dispensed into the chosen target plate format.

System Description

The Genomic STARlet is equipped with either four or eight independent pipetting channels. Loading of the deck with carriers containing tips, reagents, 96-well PCR microplate(s) and NucleoFast® 96 PCR Plates can either be done manually or automated if the Autoload is present. The system is equipped with a CVS vacuum station to perform the filtration procedure. The plate movements during the process are performed by the CO-RE Gripper.

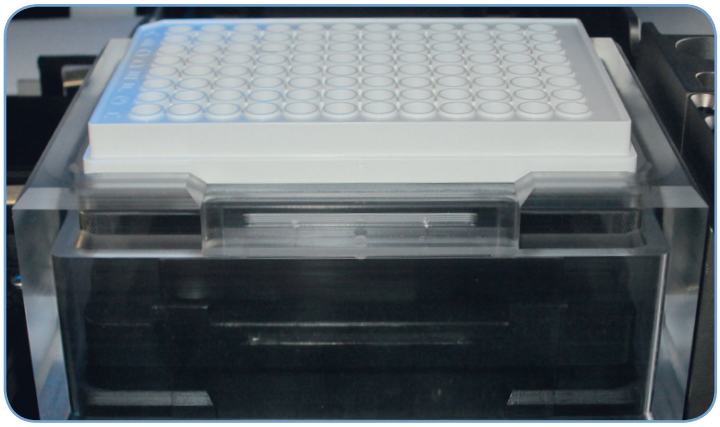

Figure 3: NucleoFast® 96 PCR Plate on the CVS.

Verification

Automation of the MACHEREY-NAGEL NucleoFast® 96 PCR kit has been verified on the Genomic STARlet. A method has been developed which includes the entire automated process for one 96-well PCR plate. When applying the verified method the user is only required to load and unload the labware carriers containing plates and tips. The PCR products ranging from 164bp up to 1484bp were automatically purified on the Genomic STARlet using the method described above. The PCR products were analysed using capillary electrophoresis on the 2100 Bioanalyzer (Agilent Technologies). Efficiency (recovery rate) and DNA quality of the purified PCR products were assessed.

Results

Eight PCR fragments of 164, 252, 359, 459, 645, 782, 982 and 1,484bp in lengths were amplified in 12 wells of a 96-well PCR plate each. Two individual purification runs were performed.

DNA quality & recovery rate

The quality of the purified PCR samples was analysed by capillary electrophoresis (Figure 4A,B) The analysed fragments of all sizes show in both independent purifications sharp bands of equal size compared with an aliquot of unpurified PCR product. This indicates that no degradation has occured during the process. The recovery rate depends on the length of the purified PCR products. Figure 5 shows that recovery is excellent (>80%) for fragments larger than 250 bp, while it is still good (70%) for small products down to 150 bp. After purification, DNA concentrations of all PCR fragments of the PCR plate were analysed by PicoGreen measurement. For each fragment 12 wells were analysed, avarage and deviation of resulting yields was determined (Figure 6). Homogeneous results and a high reproducibility of the automated PCR purification process performed on the Genomic STARlet over the entire NucleoFast® PCR Plate is demonstrated for all fragment lengths.

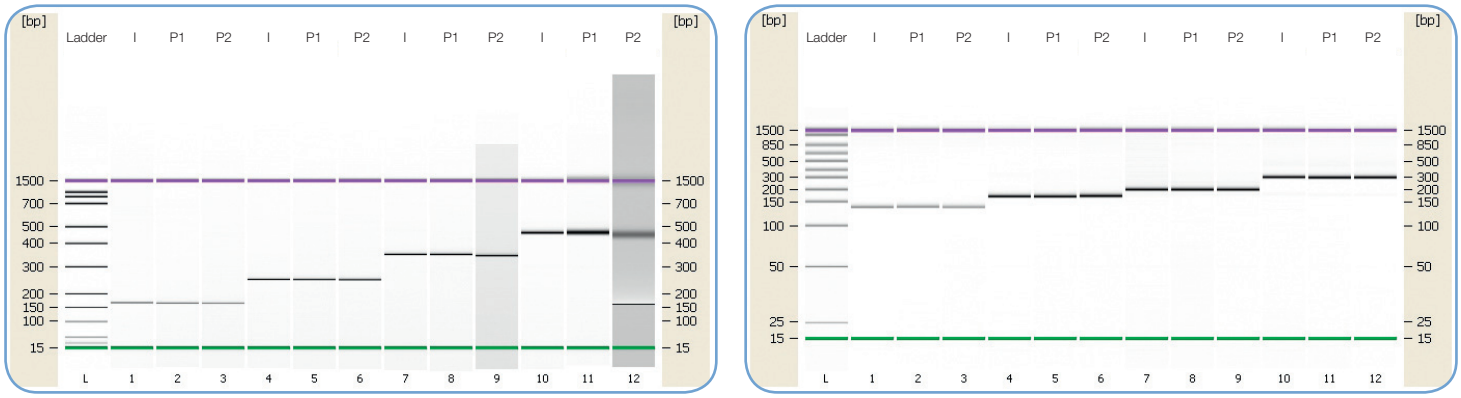

Figure 4A,B: PCR fragment analysis on the 2100 Bioanalyzer for determination of DNA purity. An aliquot of each PCR reaction was analyzed before (I = input) and after (P1 = purification 1, P2 = purification 2) two independent purification procedures. (A) Results from PCR fragments of 164bp (lane 1-3), 252 bp (lane 4-6), 359bp (lane 7-9) and 459bp (lane 10-12) length. (B) Results from PCR fragments of 645bp (lane 1-3), 782bp (lane 4-6), 982bp (lane 7-9) and 1482bp (lane 10-12) length.

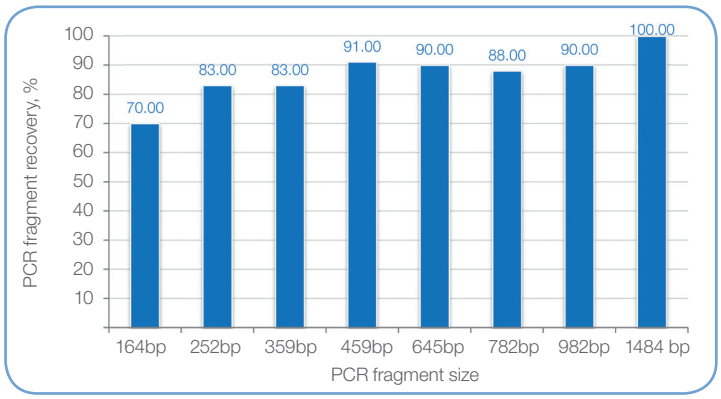

Figure 5: DNA recovery rate for the eight PCR fragments, determined in two independent purification runs.

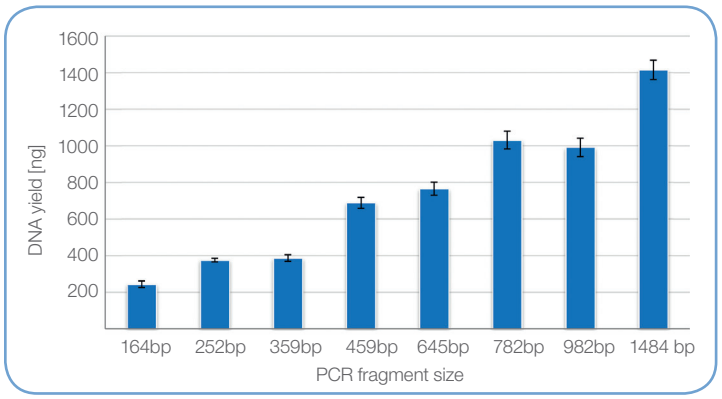

Figure 6: DNA yield for the 8 individual PCR fragments. Means & standard deviations from 12 wells are shown. 25µl of each PCR fragment were purified (n=12, each fragment), purified fragments were recovered in 120µl recovery buffer (120µl dispensed, 115µl recovered).

Throughput and capacity

When using a Genomic STARlet equipped with four pipetting channels, the purification of 96 PCR samples with the NucleoFast® 96 PCR kit was completed in 75 minutes. Processing time can be reduced down to 30 minutes when using eight pipetting channels. Tip consumption is very economical with 2.13 tips per sample for the four channel configuration and 2.08 tips per sample for the eight channel configuration.

Discussion

Hamilton and MACHEREY-NAGEL have developed and verified a very fast and cost efficient solution for fully automated PCR purification with maximum throughput, recovery and reliability. The ultrafiltration principle of the NucleoFast® 96 PCR kits could be automated easily on the Genomic STARlet. A 96-well PCR plate can be processed within 30 minutes without user intervention when using the eight channel configuration. The ultrafiltration membrane of the NulceoFast® 96 PCR Plate is insensitive to mechanical disruption by tips, it is detergent free and produces pure, ready-to-use DNA.

Typical downstream applications are Sanger (cycle) sequencing, PCR-based labelling, labelling PCR products with radioactive or non-radioactive markers by Klenow reactions, microarray analysis, ligation and transformation of PCR products, restriction digestion for qualitative and quantitative analysis, in vitro transcription using the PCR products as a template or microinjection for gene transfer between animals.

System Requirements	Part Number
Genomic STARlet, 4 channels, manual load, CVS vacuum station, Hamilton Heater Shaker, classic life science package	806200
Genomic STARlet, 8 channels, manual load, CVS vacuum station, Hamilton Heater Shaker, classic life science package	806210
Genomic STARlet, 4 channels, Autoload, CVS vacuum station, Hamilton Heater Shaker, classic life science package	806220
Genomic STARlet, 8 channels, Autoload, CVS vacuum station, Hamilton Heater Shaker, classic life science package	806230

System Dimensions

Width: 1124mm, Height: 903mm, Depth: 795mm (Autoload: 1006 mm)

Labware requirements, kits	Size	Part number / Provider
NucleoFast® 96 PCR	4 x 96	743500.4 / MACHEREY-NAGEL
	24 x 96	743500.24 / MACHEREY-NAGEL
	10 x 96	743500.10 / MACHEREY-NAGEL
	50 x 96	743500.50 / MACHEREY-NAGEL

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