Fast Automated PCR Purification with Ultrafiltration Technology on the MICROLAB® STAR

Speed and cost are a major issue with frequently used PCR purification applications. Based on the NucleoFast® technology from MACHEREY-NAGEL, HAMILTON now offers an automated solution for nucleic acid purification on the liquid handling robot MICROLAB® STAR that is guaranteed to save time and money. Using dependable vacuum technology, contaminants are removed by means of ultrafiltration - without the need of binding and elution steps. The throughput of this validated solution is 96 samples in 25 minutes. Up to four 96-well plates can be run without user intervention.

Equipment and Materials

Equipment

HAMILTON's validated standard application "MN PCR Purification with Ultrafiltration" (P/N 187004) includes:

- MICROLAB® STAR, 8 channels, with built-in robotic plate-handler (iSWAP), manual load
- MICROLAB® BVS Basic Vacuum System incl. ME 4C Vario Membrane Pump and CVC 2000 Controller (Vacuubrand GmbH, Wertheim, Germany)
- MICROLAB® STAR Shaker (Variomag® Teleshake, H+P Labortechnik, Oberschleissheim, Germany)
- All required carriers, and the complete method

Chemicals

- NucleoFast® 96 PCR Purification Kit (from MACHEREY-NAGEL GmbH, Düren, Germany)

Figure 1: *The internal plate handler iSWAP places the NucleoFast® 96 PCR plate on the vacuum chamber of the MICROLAB® BVS (Basic Vacuum System), which is positioned on the MICROLAB® STAR deck.*

Protocol

Deck Layout

The deck is manually loaded with carriers containing tips, reagents, 96-well PCR microplate(s) and filter plates. The MICROLAB® BVS (Basic Vacuum System) and the MICROLAB® STAR shaker are mounted on a carrier that is fixed to the deck. The plate movements during the process are performed by the iSWAP robotic plate-handler (Figure 1).

Application Software

The validated method was developed with MICROLAB® Vector software. It includes the method itself, labware definitions and liquid classes.

Method

The PCR reactions are adjusted to 100 µl volume with sterile water and transferred to the NucleoFast® 96 PCR filter plate. The PCR products are collected on the surface of the ultrafiltration membrane, while contaminants are filtered out to waste, in a vacuum created by the MICROLAB® BVS. A wash step with 100ul sterile water is optional. The purified PCR products are recovered from the membrane after addition of 50–100 µl of RB buffer or sterile water and a short incubation period of 1 minute. The filter plate is slightly shaken during the incubation.

Validation

The MICROLAB® STAR is validated for the automation of the MACHEREY-NAGEL NucleoFast® 96 PCR kit. The validated system includes the instrument, the labware carriers and the software. The user is only required to load and unload the labware carriers.

Results

96 PCR samples, ranging from 164 bp to 1,484 bp, were purified on the MICROLAB® STAR using the NucleoFast® 96 PCR kit and the method described above. The PCR products were analysed on 1% agarose gels after purification. Recovery, throughput and quality were assessed.

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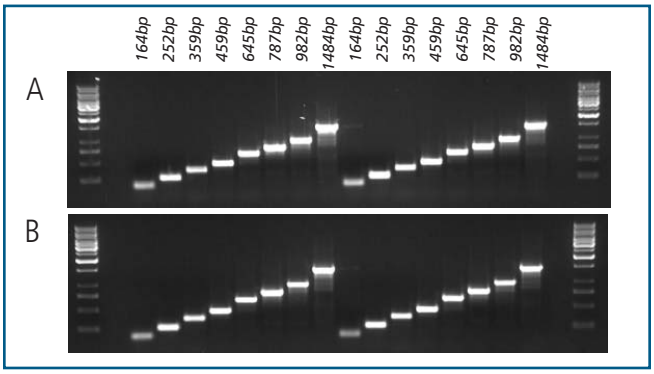

Figure 2: *PCR fragments of different length are purified with the NucleoFast® 96 PCR kit based on the ultrafiltration technique. The PCR fragments are eluted with 100µl elution buffer. After concentration, the samples are separated by electrophoresis on 1% agarose gel for qualitative comparison. The results obtained over two different runs (A,B) are homogenous .*

Homogenous DNA quality and reproducible DNA recovery rates

Purified PCR samples of different length were analysed on a gel for qualitative comparison (Figure 2). Homogenous results were obtained over different runs and no contamination was observed.

The recovery rate depends on the length of the purified PCR products (Figure 3). The rate is high, with 70% or more for fragments larger than 250 bp, and still good for small products down to 150 bp.

Samples processed with the MICROLAB® STAR are clean and ready to use in downstream applications, such as analysis on a sequencer (Figure 4).

Throughput and Capacity

The purification of 96 PCR samples with the NucleoFast® 96 PCR kit was completed in 25 minutes. Up to four 96-well plates can be processed in 1 hour and 40 minutes without user intervention. Deck capacity - and therefore walk-away time - may be increased by integrating additional plate stackers.

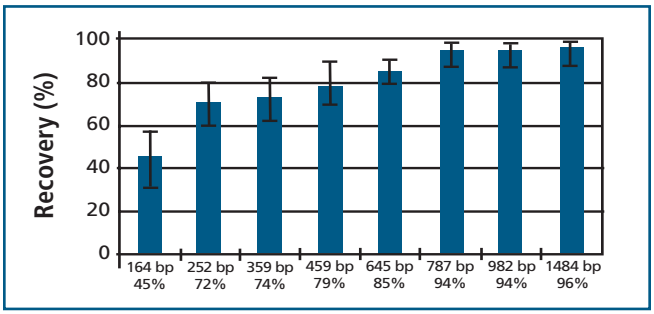

Figure 3: *Average DNA recovery rates of the purified PCR fragments with different lengths. The recovery rate depends on the length of the products. But even for small fragments (164 bp), the recovery rates are good (45%). The average and standard deviation values were determined from 12 parallel purifications per fragment size (96 in total).*

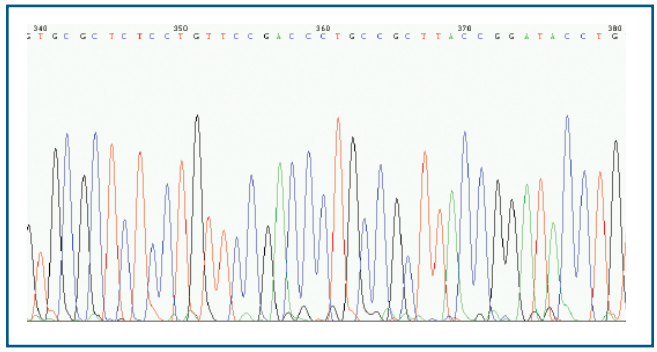

Figure 4: *Part of a sequence from a 982 bp PCR fragment, which was purified with the NucleoFast® 96 PCR kit. The automated fluorescent sequencing using standard BigDye-terminator chemistry was done with an ABI PRISM 3700 DNA analyser.*

Discussion

HAMILTON and MACHEREY-NAGEL have developed a validated method for fully automated PCR purification with maximum throughput and reliability. The incubation of the elution buffer is processed on the shaker in order to increase the DNA recovery rate. The highly flexible system provided by HAMILTON can be adapted to other MACHEREY-NAGEL kit types. Further options such as DNA normalization, PCR preparation and DNA digestion are also available.

Features and Benefits

- Fully automated hands-free processing with built-in robotic plate-handler (iSWAP)
- Processing of up to four 96-well PCR plates without user intervention
- Fast purification of 96 samples within 25 minutes
- High DNA recovery over a broad range of PCR fragment length down to 150 bp
- Validated method available
- Process control and security with MICROLAB® BVS (Basic Vacuum System)
- A wide range of MACHEREY-NAGEL kit types can be automated on the same platform
- Automation of additional applications like PCR preparation or DNA digestion

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