Genomics

Automated PCR Clean-Up with Silica on the MICROLAB® STAR

PCR preparation and purification are standard procedures used for genetic analysis. Based on the widely accepted and reliable vacuum technology, Hamilton offers an automated solution for PCR clean-up on the MICROLAB® STAR liquid handling robot featuring the NucleoSpin® Robot-96 Extract kit from MACHEREY-NAGEL. The throughput of this validated solution is 96 samples per hour.

Equipment and Materials

HAMILTON validated standard application MN PCR Clean-up with Silica (P/N 187003) including:

Equipment

- MICROLAB STAR®, 8 channels, with built in robotic plate-handler (iSWAP), manual load
- MICROLAB® AVS Automated Vacuum System
- All required carriers, and the complete method

Chemicals

- NucleoSpin® Robot-96 Extract kit (MACHEREY-NAGEL GmbH, Düren, Germany)

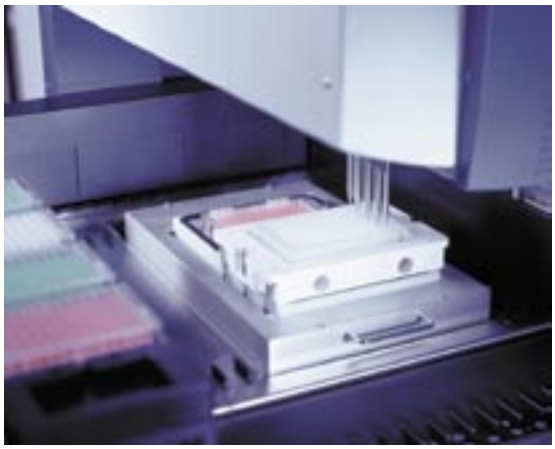

Figure 1: *MICROLAB® AVS (Automated Vacuum System) with two chambers and movable carriage built into the MICROLAB® STAR deck.*

Protocol

Deck Layout

The deck is manually loaded with carriers containing tips, reagents, 96-well PCR microplate(s) and filter plates. The MICROLAB® AVS (Automated Vacuum System) is built into the deck of the MICROLAB® STAR (Figure 1). The plate movements during the process are performed by the iSWAP robotic plate-handler (Figure 2).

Figure 2: *The internal plate handler iSWAP can also reach positions outside of the MICROLAB® STAR deck.*

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 100ul volume, mixed with NTB binding buffer and transferred to the NucleoSpin® Extract Binding plate. The DNA binds to the silica matrix as the samples pass through the plate in a vacuum created by the MICROLAB® AVS. The samples are then washed with NT3 buffer and dried by full vacuum application. The AVS disposes of the waste and once the plate is free of ethanol, the AVS positions the NucleoSpin® plate for sample elution.

HAMILTON

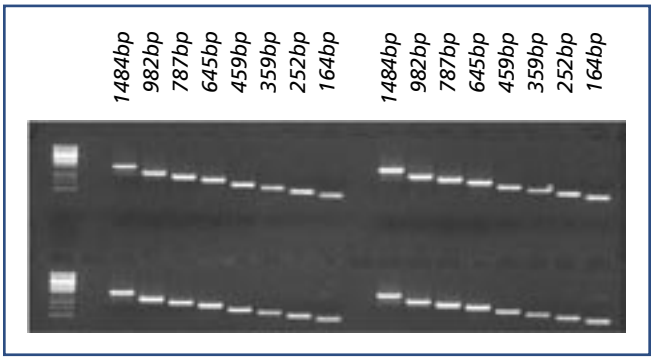

Figure 3: *A set of different PCR fragments were purified with the NucleoSpin® Robot-96 Extract kit. The purified PCR fragments were eluted with 125µl elution buffer, concentrated, and analysed by electrophoresis on 1% agarose gel. Homogenous results were obtained.*

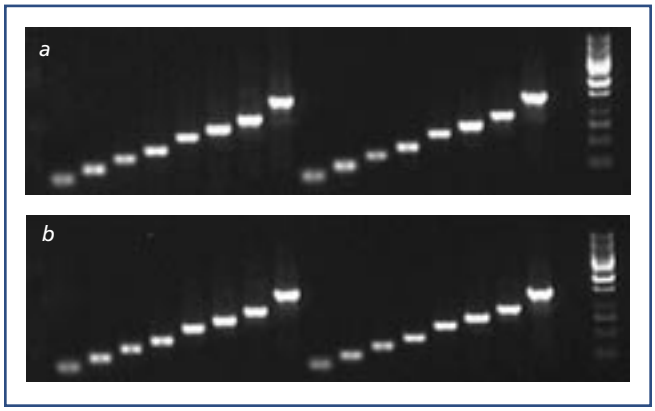

Figure 4: *Qualitative comparison of PCR products with different lengths (a) before and (b) after purification.*

Validation

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

Results

96 PCR samples, ranging from 164bp to 1484bp, were purified on the MICROLAB® STAR using the NucleoSpin® kit and the method described above. After purification, the PCR products were analysed on 1% agarose gels. Recovery, throughput and evidence of carryover were assessed.

Consistent Performance

The automated process on MICROLAB® STAR provided consistent purification of PCR fragments of different sizes (164bp to 1484bp) (Figure 3). There was no significant loss of product due to the purification process. This is demonstrated by qualitative comparison of the samples before and after purification (Figure 4). Furthermore, there was no evidence of carryover, as the absence of cross-contamination bands on the gel from samples located in adjacent wells indicates.

Throughput and Capacity

The NucleoSpin® kit was completed in 60 minutes for 96 samples. This system can process up to two 96-well PCR plates in 2 hours without user intervention. Additional plate stackers may be integrated to increase deck capacity and walk-away time.

Discussion

HAMILTON together with MACHEREY-NAGEL have developed and validated a method for fully automated PCR purification with maximum reliability and excellent DNA recovery. The very flexible system with the automated vacuum box from HAMILTON can be adapted to run other MACHEREY-NAGEL kit types. HAMILTON's unique CO-RE (Compression-induced O-Ring Expansion) technology for tip coupling on the pipetting channels of MICROLAB® STAR allows the use of both disposable tips and washable needles within this application.

Features and Benefits

- Fully automated hands-free processing with built-in robotic plate-handler (iSWAP)
- High DNA recovery over a broad range of PCR fragment length
- Process control and security with MICROLAB® AVS – automated vacuum system
- Validated method procedure available
- Automation of multiple genomic applications including DNA normalization, PCR setup and restriction analysis
- A wide range of MACHEREY-NAGEL kit types can be automated on the same platform

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