

Genomics



Automated Plasmid Purification on the MICROLAB® STAR

Flexible and efficient workstations for isolation of DNA have become more and more important in the past few years in many areas of life sciences, as sample preparation is often a prohibitively time-consuming step in genetic analysis. As a solution to this problem, HAMILTON now offers a highly flexible purification system on the liquid handling robot MICROLAB® STAR (Figure 1) for the NucleoSpin® Robot-96 Plasmid Purification Kit from MACHERY-NAGEL. The system processes 96 samples within 75 minutes.

Equipment and Materials

Equipment

HAMILTON validated standard application MN Plasmid Purification EU, 230V (P/N 187002) or MN Plasmid Purification US, 115V (P/N 187006) including:

- MICROLAB® STAR, 8 channels, with built-in robotic plate-handler (iSWAP), manual load
- MICROLAB® AVS Automated Vacuum System
- MICROLAB® STAR Shaker (Variomag® Teleshake, H+P Labortechnik, Oberschleissheim, Germany)
- All required carriers, and the complete method

Chemicals

- NucleoSpin® Robot-96 Plasmid Purification Kit (MACHERY-NAGEL GmbH, Düren, Germany)

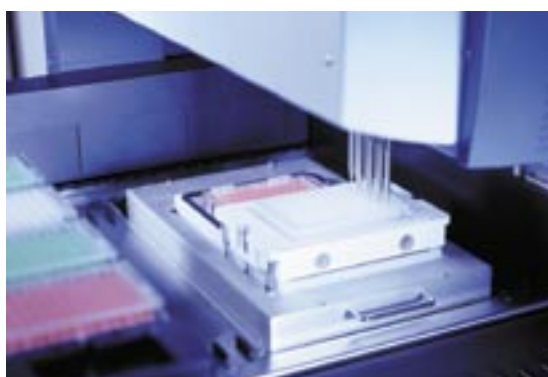


Figure 1: MICROLAB® AVS (Automated Vacuum System) with two chambers and movable carriage built into the MICROLAB® STAR deck.

Protocol

Deck Layout

The deck is manually loaded with carriers containing tips, a deep-well plate with pelleted bacterial cultures, reagents, microplates and filter plates. The MICROLAB® AVS (Automated Vacuum System) is built into the deck of the STAR. The plate movements during the process are performed by the iSWAP robotic hand.

Application Software

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

Method

The method involves four basic steps, including bacterial lysis, DNA binding to the silica matrix, washing and elution. The pelleted cells are resuspended with the integrated Variomag Teleshake shaker. The cells are then lysed, neutralized and transferred to the NucleoSpin filter plate on the MICROLAB® AVS. The filtered lysate passes directly into the NucleoSpin® binding plate and the plasmid DNA binds to the silica matrix as the sample flows through the plate with applied vacuum pressure. The lysate clearing plate is removed by the iSWAP and the NucleoSpin® plate is washed and dried. The waste from the lysate clearing and washing stages is automatically evacuated by the AVS. Once the NucleoSpin® plate is dry, it is transferred to the elution chamber of the vacuum box and the plasmid DNA is eluted.

Validation

The MICROLAB® STAR is validated for the automation of the MACHERY-NAGEL NucleoSpin® 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

Plasmids with four different inserts were purified with the NucleoSpin® Robot-96 kit on the MICROLAB® STAR. The cultures containing these four vectors were distributed in a staggered pattern throughout the 96 deep-well plate for overnight incubation and processing. The yield, quality and evidence of carryover were assessed.



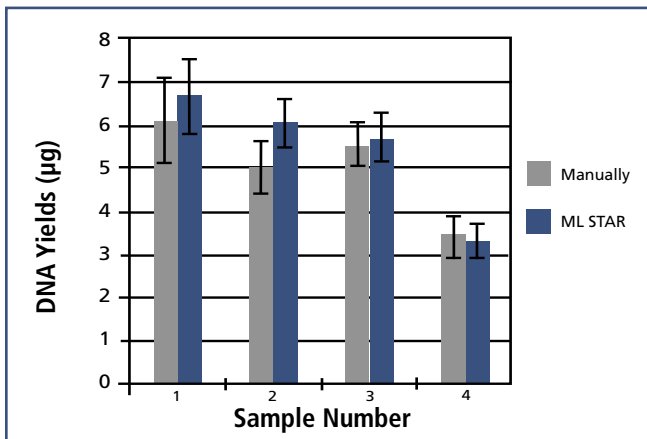


Figure 2: pGEM plasmid yields from manual and automated preparation. Average values and standard deviation values were determined from 24 parallel isolations per plasmid (96 in total).

Yield and Quality

The yield obtained by automated processing using the MICROLAB® STAR was compared with the yield from manual processing. The yields ranged from approximately 3µg to 6.5µg, depending on the purified plasmid. The yields from the automated system were consistent with, if not greater than the yields from manual processing (Figure 2). The A260/280 ratios of the processed DNA for both the manual and automated systems were all within optimal ranges (Figure 3). The samples processed with the MICROLAB® STAR were consistent, clean and ready to use in downstream applications, such as digestion with restriction enzymes (Figure 4).

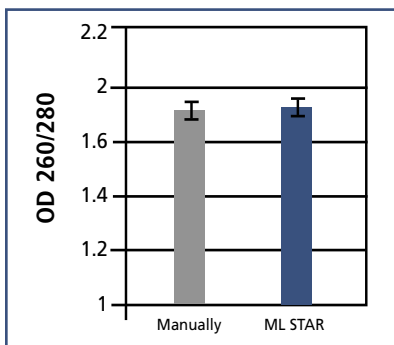


Figure 3: The A260/280 ratio for the purified plasmids from 96 isolations in total. The A260/280 ratio for both the manually and automatically prepared samples was within the optimal range.

Carryover

Figure 4 shows results from the isolation. No cross-contamination of plasmids from adjacent wells is visible on the gel. In order to further test for carryover, each purified sample was PCR-amplified with vector-specific primers. No carryover was seen (data not shown).

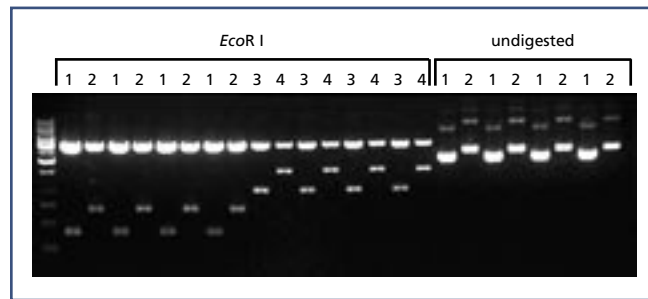


Figure 4: Purified plasmid DNA (pGEM with insert sizes of 359bp (1), 645bp (2), 982bp (3), and 1484bp (4)). Different plasmids were located in adjacent wells of the plates. Randomly-selected plasmids of all insert sizes were digested with EcoR I. Digested and undigested plasmids (10µl) were run on a 1% agarose gel.

Throughput and Capacity

The NucleoSpin® kit process was completed in 75 minutes for one 96-well plate. This system can process up to two 96-well plates in 2 hours and 30 minutes without user intervention. The iSWAP is used for removing the NucleoSpin binding plate and the MN wash plate from the AVS prior to the elution step. Additional plate stackers may easily be integrated to increase deck capacity and walk-away time.

Discussion

HAMILTON and MACHERY-NAGEL have developed a validated method for fully automated plasmid purification with maximum reliability, yield and throughput. MICROLAB® STAR with the flexible AVS vacuum manifold can also be used to automate other MACHERY-NAGEL vacuum separation kits. 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 yield and DNA quality
- Validated method available
- Process control and security with MICROLAB® AVS (Automated Vacuum System)
- Automated additional applications, such as DNA normalization, PCR set up, or restriction analysis
- A wide range of MACHERY-NAGEL kit types can be automated on the same platform

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