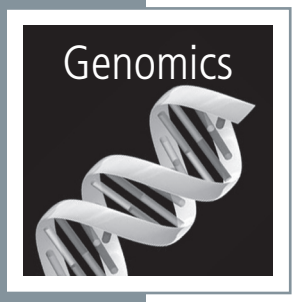

Automated Plasmid Purification on the Genomic STARlet

Flexible and efficient workstations for isolation of DNA have become more important in the past few years in many areas of life sciences, as sample preparation is a time-consuming step in genetic analysis. As a solution to this problem, HAMILTON and MACHEREY-NAGEL have designed and validated the Genomic STARlet for different applications, e.g. plasmid DNA purifcation. The flexible purification system is based on the liquid handling robot MICROLAB® STAR suitable for use of the silica membrane based NucleoSpin® 8/96 Plasmid Purification Kits from MACHEREY-NAGEL.

System Requirements		Part Number
Genomic STARlet, 4 channels, CVS Vacuum Station, HAMILTON Heater Shaker, Classic Life Science Package		806200
Genomic STARlet, 8 channels, CVS Vacuum Station, HAMILTON Heater Shaker, Classic Life Science Package		806210
Labware Requirements, Kits	**Size**	**Part Number**
NucleoSpin® 8 Plasmid	12 x 8	740621
	60 x 8	740621.5
NucleoSpin® 96 Plasmid	1 x 96	740625.1
	4 x 96	740625.4
	24x96	740625.24

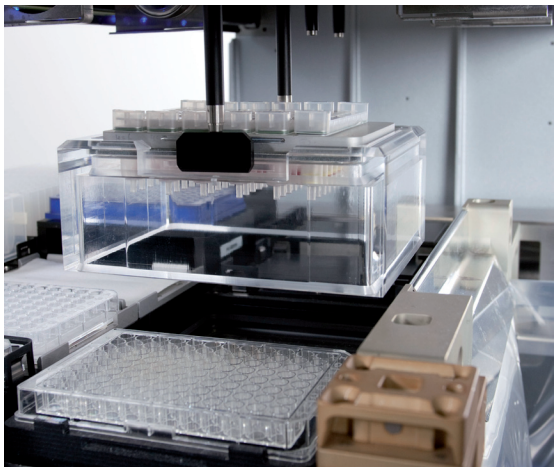

Figure 1: Transport of CVS vacuum manifold loaded with 8-well NucleoSpin® Blood Binding strips on the adapter plate using the HAMILTON CO-RE Gripper.

Protocol

The deck is manually loaded with carriers containing tips, reagents, filter plates and a culture plate or culture tubes with the bacteria samples. A MICROLAB® CVS Vacuum module and a HAMILTON Heater Shaker are integrated on the deck. The plate movements as well as the loading and unloading of the vacuum box during the process are performed by the CO-RE Gripper (Figure 1). The Genomic STARlet is controlled by the MICROLAB® VENUS ONE software. A dedicated application interface leads the user through the isolation process. Further application relevant parameters (e.g. vacuum settings, filtration times, optional wash step) can easily be adjusted by the user.

Method Description

The four basic steps to purify plasmid DNA are: bacterial cell lysis, bacterial lysate clearing step, DNA binding step, washing steps, and elution of the purified plasmid DNA. The culture plate / or tubes with the pelleted bacterial cells are loaded to the deck; buffer is added to the pellets and bacterial cells are resuspended using the integrated HAMILTON Heater Shaker. The cells are then lysed, neutralized and transferred to the NucleoSpin® NucleoSpin® Plasmid Filter Strips or the NucleoSpin® Plasmid Filter Plate - on the MICROLAB® CVS. By applying vacuum pressure, the crude bacterial lysates are filtered through the NucleoSpin® Filter strips or plate. Cleared lysate is collected directly in the NucleoSpin® Plasmid Binding Strips or Plate and after rearrangement of the vacuum manifold, plasmid DNA binds to the silica membrane in a second filtration step. Contaminants are removed in a subsequent washing step. The use of a MN Wash Plate reduces ethanol contamination during the washing steps. The MN Wash Plate is removed by the CO-RE Gripper and the NucleoSpin® Plasmid Binding Strips or Plate are dried by applying vacuum. Finally, purified plasmid DNA is eluted in either a standard microtiterplate or in a rack with elution tubes.

HAMILTON

Validation

The isolation of plasmids was validated with pelleted bacteria on the Genomic STARlet. The pelleted bacteria were loaded in 96-well culture plates to the deck and processed as described above. 8-well strips or a 96-well binding plate were used for the isolation. The obtained yield and purity were assessed on a Biotek Lambda scan 200 reader and on agarose gels (1% TAE). The results were compared with samples isolated manually. For sequencing, the samples were sent to MWG-Eurofins (Germany).

Results

48 and 96 samples were processed both manually and automated on the Genomic STARlet with the 8-well strips and the 96-well binding plates, respectively. Figure 2 summarizes the obtained purities and yields of the plasmids. The yields were between 9.75 µg and 10.60 µg (Fig. 2A) and the purities were between 1.86 and 1.90 for all samples (Fig. 2B). Additionally, the analysis on agarose gels revealed and confirmed high purity of the samples (results not shown). High quality sequencing data were obtained for up to 900 bp (Fig. 3).

The Genomic STARlet processed 48 samples with the 8-well strips or 96 samples with the 96-well binding plate in less than 80 minutes.

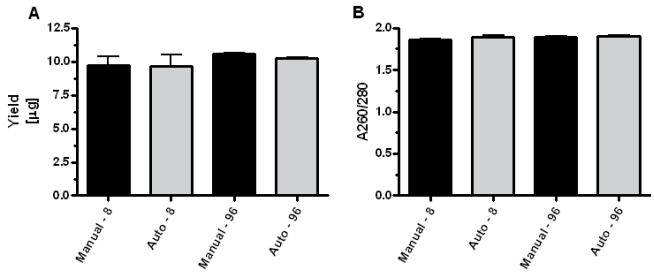

Figure 2: Plasmid yields (A) and purities (B) after the isolation from pelleted bacteria. 8: samples were extracted with 8-well strips. 96: samples were extracted with 96-well binding plate. Standard deviations are shown.

Discussion & Summary

The Genomic STARlet has been validated for the isolation of plasmids from bacteria. The yields and purities from automated and manual isolation processes showed similar results and were in the expected range. The isolated plasmids could be used directly in a downstream application such as sequencing. In summary, the automation of the process increases standardization by reducing errors. Further, flexible numbers of samples can be processed and additional extraction assays, e.g. PCR purifications, can be performed on the same system. This makes the Genomic STARlet a powerful and versatile device in a classic life science laboratory.

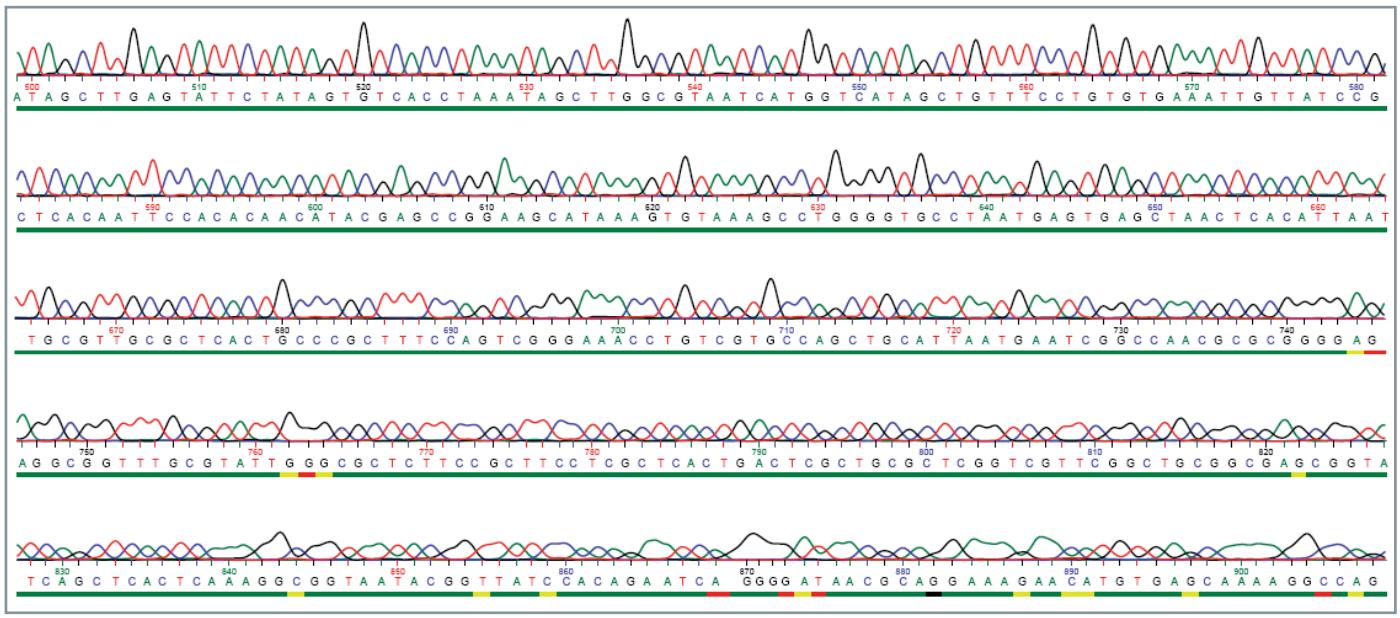

Figure 3: Partial sequencing data from a plasmid sample isolated by using the 8-column strip. A high quality sequence was obtained for up to 900 bp.

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