Fully Automated Genomic DNA Isolation from Blood on the MICROLAB® STAR

Reliability and process control are major issues for automated genomic DNA isolation from blood samples. The new automated solution from HAMILTON on the MICROLAB® STAR liquid handling robot using NucleoSpin® technology from MACHEREY-NAGEL features blood clot detection during aspiration and a clog check of the filtration plates after each vacuum step. Our monitored air displacement pipetting principle allows the detection of incorrect aspiration, such as clots in blood samples. After each vacuum step, the filter plates are checked for clogs using our advanced liquid level detection system with both pressure and capacitive LLD. Barcode identification and sample tracking are available options. The system processes 96 samples within 60 minutes.

Equipment and Materials

Equipment

HAMILTON's validated standard application MN gDNA Isolation from Blood includes:

- MICROLAB® STAR, 8 channels, with built-in robotic plate-handler (iSWAP), manual or autoload
- 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

Reagents

- NucleoSpin® 96 Blood (from MACHEREY-NAGEL GmbH, Düren, Germany)

Protocol

Deck Layout

The deck is manually loaded with carriers containing tips, reagents, blood samples, microplates and filter plates. The blood samples can either be loaded in tubes or in microtiter plates. Automatic loading of the samples including barcode reading is an option. 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.

Application Software

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

Method

Proteinase K and up to 200µl blood from each sample are transferred to the lysis block. After the addition of the lysis buffer, the samples are incubated and shaken for 10 minutes at room temperature on the Variomag Teleshake. Ethanol is added to the crude lysates and mixed well before the samples are transferred to the NucleoSpin® Blood binding plate. An overlay with wash buffer prevents foaming during the filtration step, in a vacuum created by the MICROLAB® BVS. After the binding of the DNA, the silica membranes are washed three times with two different wash buffers. Once the NucleoSpin®plate is dry, the very pure genomic DNA is eluted with 50-200µl buffer. As an option, a clog check for the filter plates after every vacuum step can be selected.

Validation

The MICROLAB® STAR is validated for the automation of the MACHEREY-NAGEL NucleoSpin® 96 Blood 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.

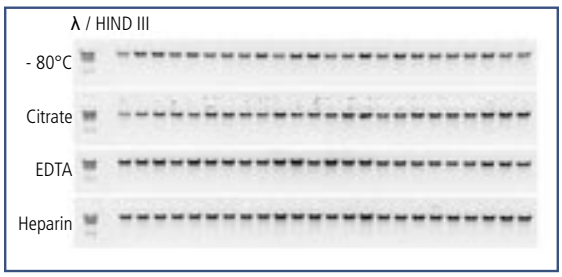

Figure 1: *A total of 96 isolated genomic DNA samples with the NucleoSpin® 96 Blood kit from differently stabilized and stored blood samples. 10µl of 200µl eluted gDNA from each sample were separated by electrophoresis on 1.0% agarose gel for qualitative comparison. The results obtained are very homogenous, independently of the procedure used for storing the blood samples.*

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Results

Differently stabilized blood samples were purified on the MICROLAB® STAR using the NucleoSpin® 96 Blood kit and the method described above. The isolated genomic DNA was analysed on agarose gels and with absorption readers. The quality, reproducibility and evidence of carry-over were assessed.

Homogenous DNA quality and yields

Isolated genomic DNA samples were analysed on a gel for qualitative comparison (Figure 1). Homogenous results were obtained, independently of whether the blood samples used for the isolation were stored at –80°C or stabilized either with citrate, EDTA or heparin and stored at 4°C.

The A260/280 ratio for the isolated genomic DNA was within the optimal range of ≥1.8 – 2.0. PCR inhibitors (citrate, EDTA, heparin) are completely removed. Samples processed with the MICROLAB® STAR are ready to use in downstream applications, such as PCR analysis with the LightCycler® from Roche (Figure 2).

Carry-over

To test for carry-over, the blood samples were distributed in a chequerboard pattern throughout the 96-well lysis block for genomic DNA isolation. No cross- contamination was detectable with PCR amplification (Figure 3).

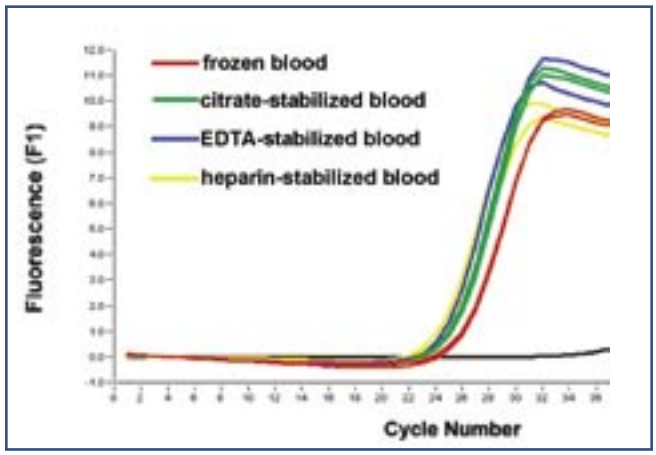

Figure 2: *PCR analysis of the isolated genomic DNA samples with the LightCyclerTM (Roche). LightCycler® amplification shows efficient removal of PCR inhibitors (haeme, citrate, EDTA or heparin). DNA was extracted from samples (see Figure 1) and subjected to LightCycler® amplification of a 200bp ß-actin fragment (SYBR Green detection). All samples show similar CT (Threshold Cycle) values.*

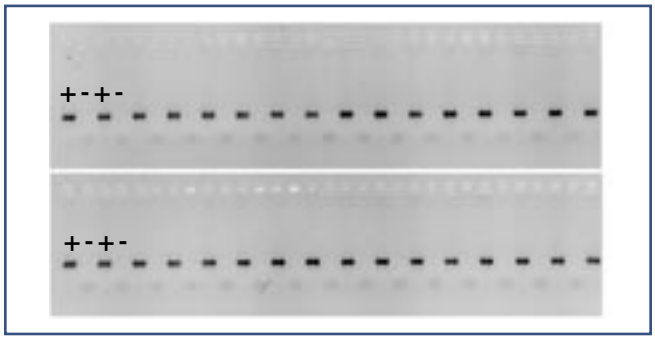

Figure 3: *No cross-contamination was detectable with PCR amplification. The blood samples were distributed in a chequerboard pattern throughout the 96-well lysis block for DNA isolation. The PCR products of 32 samples (ß-actin PCR, 35 cycles), separated by electrophoresis on agarose gel, are shown in the picture.*

Throughput and Capacity

The isolation of 96 blood samples with the NucleoSpin® 96 Blood kit was completed in 60 minutes. This system can process up to two 96-well plates in 2 hours without user intervention. Deck capacity - and therefore walk-away time - may be increased by integrating additional plate stackers.

Discussion

HAMILTON and MACHEREY-NAGEL have developed a validated method for fully automated genomic DNA isolation out of blood samples with maximum reliability, yield and quality. Our monitored air displacement pipetting principle and advanced liquid level detection system with both pressure and capacitive LLD allows a very high degree of process security thanks to clot and clog detection. 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)
- Clog check for monitoring of the vacuum steps
- Aspiration monitoring (MAD) for blood clot detection
- Blood samples loadable in 96-well plates or tubes
- Sample tracking available as an option
- Automation of additional applications like PCR preparation or DNA digestion

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