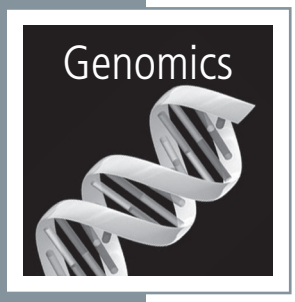

Automated genomic DNA Isolation from Blood on the Genomic STARlet

The analysis of genomic DNA from whole blood is widely used for genotyping, HLA typing, and more. It is relatively simple to draw the blood samples; the processing, however, offers some challenges due to the possible clotting of the blood. Therefore, a reliable system and process control are crucial to isolate genomic DNA from whole blood samples. (e.g., stabilized with EDTA, citrate or heparin). HAMILTON and MACHEREY-NAGEL have developed and validated the Genomic STARlet to isolate genomic DNA from blood samples using the NucleoSpin® technology. This application note describes the automated isolation of genomic DNA from blood and compares the results with a manual isolation process. Further, it shows how HAMILTON's monitored air displacement pipetting principle allows the detection of erroneous pipetting steps and helps to increase the process stability.

System Requirements		Part Number
Genomic STARlet, 4 channels, CVS Vacuum Station, HAMILTON Heater Shaker, Genomics package		806202
Genomic STARlet, 8 channels, CVS Vacuum Station, HAMILTON Heater Shaker, Genomics package		806212
Labware Requirements, Kits	**Size**	**Part Number**
NucleoSpin® 8 Blood Containing 8-well strips	12 x 8	740664
	60 x 8	740664.5
NucleoSpin® 96 Blood Containing 96-well plates	2 x 96	740665.1
	4 x 96	740665.4
	24x96	740665.24

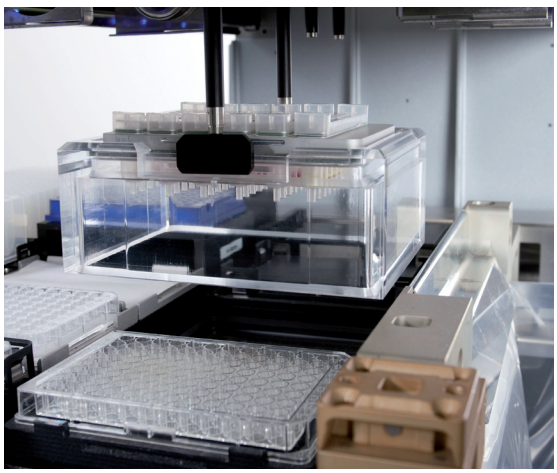

Figure 1: Transport of CVS vacuum manifold loaded with 8-well NucleoSpin® Blood Binding strips on the adapter plate using the HAMILTON CO-RE Gripper.

Protocol

The deck is manually loaded with carriers containing tips, reagents, filter plates (up to six 8-well strips mounted on an adapter plate or one 96-well binding plate) and Eppendorf or 10mL tubes with the samples. The MICROLAB® CVS Vacuum System and the HAMILTON Heater Shaker are integrated on the deck. The plate movements as well as the loading and unloading of the vacuum manifold 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 instrument setup process. Further application relevant parameters (e.g. vacuum settings, filtration times) can easily be adjusted by the user.

Method Description

Proteinase K and up to 200µl blood from each sample are transferred to the lysis block. After addition of the lysis buffer, the samples are shaken for 10 minutes at room temperature on the HAMILTON Heater Shaker. 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 following filtration step on the MICROLAB® CVS. A clog check is performed after this first vacuum filtration step and wells where clogging is detected are marked by the software and excluded from further processing. After the binding of the DNA, the silica membranes are washed three times with two different wash buffers. The NucleoSpin® Blood Binding Plate is dried under vacuum before the genomic DNA is eluted with 50-200µl elution buffer.

Validation

The isolation of genomic DNA was validated with 200µl human blood stabilized with EDTA on the Genomic STARlet. In order to evaluate the homogeneity of the purification procedure, different individual blood samples were pooled together, mixed and finally aliquots were prepared. In additional runs, the method was tested with individual samples (data not shown). 8-well strips or a 96-well binding plate were used to isolate the DNA and

HAMILTON

the obtained yield and quality were measured and compared with samples isolated manually (Biotek Lambda scan 200 Reader, qPCR by Roche LightCycler™, and 1% TAE agarose gel analysis).

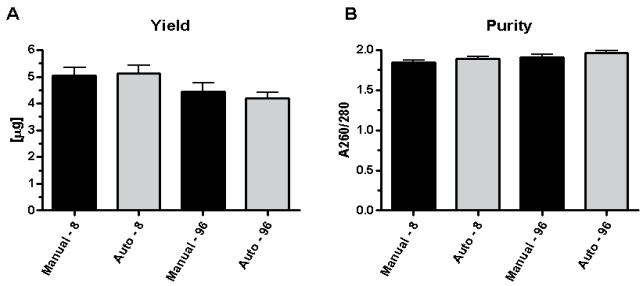

Figure 2: DNA yields (A) and purities (B) are shown after the extraction from blood. Manual and automated (Auto) processing are compared. 8 indicates that samples were extracted with 8-well strips, 96 indicates that samples were extracted with a 96-well binding plate. Standard deviations are shown.

Results

48 and 96 samples were processed both automated on the Genomic STARlet and manually with the 8-well strips and the 96-well binding plates, respectively. Figure 2 summarizes the obtained DNA purities and yields. The DNA mean yields were between 4.2µg and 5.1µg and the mean purities were between 1.84 and 1.96.

30 samples processed with 8-well strips were directly analyzed by qPCR targeting the beta-actin gene using a LightCycler™ (Fig. 3A) and showed consistent results (Cp 24.82 ± 0.15). Samples processed on the 96-well binding plate were analyzed by agarose gel electrophoresis. The isolated DNA was of high molecular weight and of a high homogenous concentration and yield (Fig. 3B). The Genomic STARlet processed 48 samples with the 8-well strips or 96 samples with the 96-well binding plates in less than 80 min.

Discussion & Summary

HAMILTON and MACHEREY-NAGEL have designed and validated the Genomic STARlet to allow optimal reliability, throughput, yield and quality for the extraction of nucleic acids from various blood samples such as fresh and frozen blood from humans or animals. Here, we demonstrate the automated isolation of genomic DNA out of human fresh blood. The automated and manual isolation resulted in similar yields and homogenous qualities of DNA. HAMILTON's pressure based total aspiration monitoring was used to detect clogging of columns. The system excluded clogged columns from further processing, thereby increasing the process stability.

Therefore, the automated isolation of DNA from blood on the Genomic STARlet assures a better standardization and consistant quality of the DNA template for downstream applications as genotyping and HLA typing.

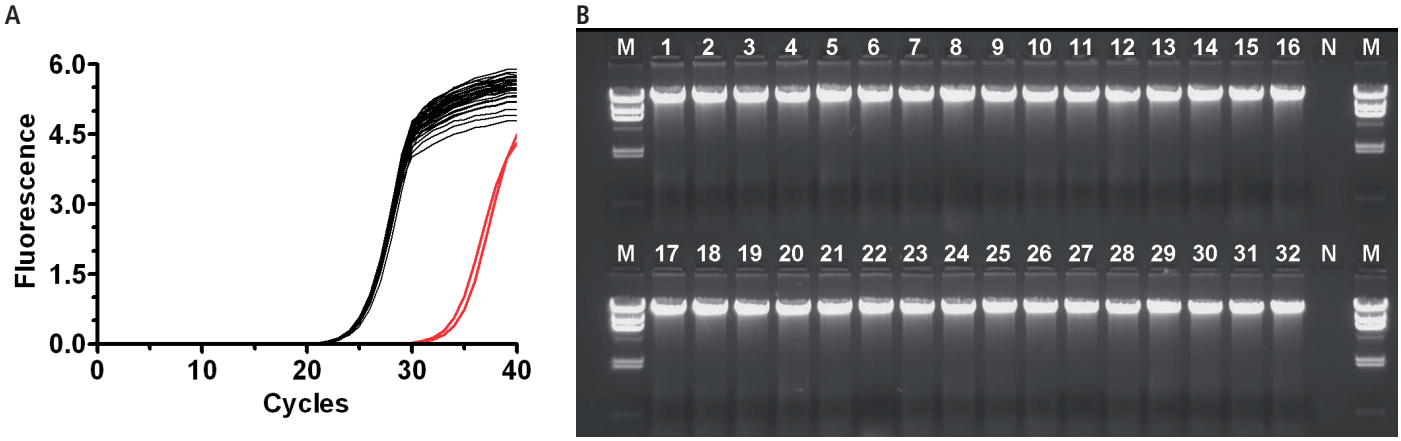

Figure 3:
A) qPCR-analysis (SYBR-green) of 30 samples eluted with 8-well strips. Cp-values of 24.82 ± 0.15 show consistent yield of isolated DNA and absence of PCR inhibitors. Black lines: Lysates. Red line: negative control.
B) 1% TAE Agarose gel shows homogenous quality of isolated DNA. 20µL eluate was loaded per lane. 1-32: samples processed with 96-well binding plate. M: λHind III size standard marker. N: empty well, no sample loaded.

MACHEREY-NAGEL

www.hamiltonrobotics.com

Lit. No. MR-1009-01/00 © HAMILTON Bonaduz AG 10/10 Printed in Germany

Headquarters
Europe - HAMILTON AG
Via Crusch 8, CH-7402 Bonaduz
Switzerland
infoservice@hamiltonrobotics.com
Americas - HAMILTON Robotics
4970 Energy Way
Reno, Nevada 89520 USA
sales@hamiltoncompany.com

United Kingdom
HAMILTON Robotics Ltd
6120 Gnd Floor Knights Court
Solihull Parkway, Birmingham
Business Park, B37 7WY, UK
Telephone: +44-121-717-0199
Fax: +44-121-717-0209
info.gb@hamiltonrobotics.com

Germany, Austria, Benelux
HAMILTON Robotics GmbH
Fraunhoferstr. 17
D-82152 Martinsried
Germany
Telephone: +49-89-5526-49-0
Fax: +49-89-5526-49-10
info.de@hamiltonrobotics.com

France
HAMILTON Robotics S.A.R.L.
Parc du Moulin de Massy
37 rue du Saule Trapu
F-91300 Massy/France
Telephone +33-1-69-75-16-16
Fax +33-1-60-11-57-16
info.fr@hamiltonrobotics.com

Italy
HAMILTON Italia Srl
Via Paracelso 22
IT-20041Agrate Brianza (MB)
Italy
Telephone +39-39-689-33-93
Fax +39-39-605-86-25
info.it@hamiltonrobotics.com

Scandinavia
HAMILTON Robotics ApS
Sankt Annæ Plads 13, 4
DK-1250 Copenhagen K
Denmark
Telephone: +45-70-26-4499
Fax +49-89-5526-49-10
Info.de@hamiltonrobotics.com

China
HAMILTON AG Shanghai Rep. Office
Room 502, No.50 Boxia RD,
Shanghai Pudong Software Prk,
Pudong, 201203 Shanghai
Telephone +86.21.6164-6567
Fax +86.21.6106-3732
info.cn@hamiltonrobotics.com