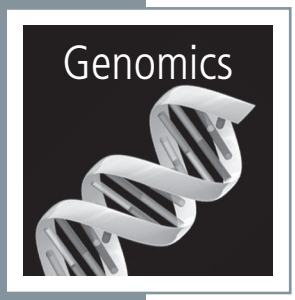

Automated genomic DNA Isolation from Mouse Tails on the Genomic STARlet

Genotyping methods like restriction-, qPCR-, and SNP-analysis are widely used in the research and diagnostic of diseases. DNA of high quality is required for a reliable analysis. HAMILTON and MACHEREY-NAGEL have developed and validated the Genomic STARlet to isolate DNA from different samples as cells and tissues using the NucleoSpin® technology. This application note describes the automated isolation of DNA from mouse tails and compares the results with a manual isolation process.

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 Tissue Containing 8-well strips	12 x 8	740740
	60 x 8	740740.5
NucleoSpin® 96 Tissue Containing 96-well plates	2 x 96	740741.2
	4 x 96	740741.4
	24 x 96	740741.24

Protocol

The deck is manually loaded with carriers containing tips, reagents, filter plates and micro plates 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.

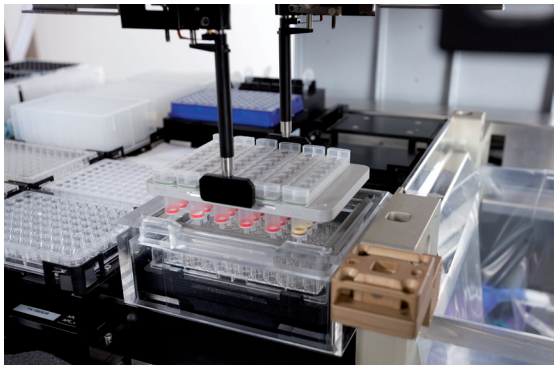

***Figure 1**: Transport of 8-well strips placed on the adapter plate to the CVS vacuum station using the Hamilton CO-RE Gripper. In the background, the HAMILTON Heater Shaker can be seen.*

Method Description

Lysates of mouse tail end clippings of about 20mg were prepared manually by shaking and incubation at 56°C overnight after the addition of lysis buffer and proteinase K. The crude lysate is loaded to the deck of the Genomic STARlet. Lysed samples are mixed with binding buffer and ethanol and transferred to the NucleoSpin® Tissue 8-well strips or a 96-well binding plate. During the following vacuum filtration step performed on the MICROLAB® CVS vacuum station, the DNA binds to the silica-membrane. A clog check is performed after this first vacuum filtration step. If a clog is detected in one of the wells the sample is excluded from further processing. The silica-membrane is washed three times with 2 different buffers and dryed under vacuum for 15min. The use of the MN Wash Plate during binding and washing steps effectively prevents ethanol contamination of the eluates by ethanolic wash buffers. Finally, the genomic DNA is eluted with pre-heated 50 – 200µl buffer.

Validation

The isolation of DNA was validated with 20mg mouse tail end clippings on the Genomic STARlet. Before loading to the deck, the mouse tails were manually prepared as described above. 8-well strips or a 96-well binding plate were used to isolate the DNA and the obtained yield and quality was measured (Biotek Lamda Scan 200 Reader and Roche LightCycler, respectively) and compared with samples isolated manually. Testing for cross contamination was performed on a 96-well binding plate by processing half of the wells with lysates and the other half with buffer only. Lysates and buffer were arranged in a checkerboard pattern as shown in Figure 4A. The processed plate was analyzed by real-time quantitative PCR (Roche LightCycler).

Results

Individual mouse tail clippings were lysed, pooled and split in aliquots in order to obtain homogenous

HAMILTON

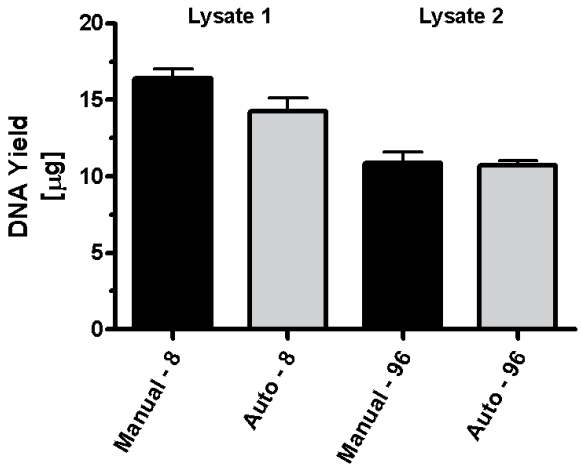

Figure 2: *DNA yields are shown after the extraction from mouse tail clipping ends. 8 indicates that samples were extracted with 8-well strips, 96 indicates that samples were extracted with 96-well binding plate. Standard deviations are shown.*

samples of master lysates. Different master lysates were used for the purification on either 8-well strips or 96-well plates. Identical samples were processed both automated on the Genomic STARlet and manually with either the 8-well strips or the 96-well binding plates, respectively. Figure 2 summarizes the obtained DNA yields. The DNA yields obtained from two different master lysates were between 10.2µg and 10.9µg for samples processed on the 96-well binding plate and between 14.3µg and 16.4µg for the samples processed on the 8-well strips. The purity was confirmed by A260/280 measurements and values between 1.8 – 1.9 were obtained. In addition, agarose gel analysis revealed high consistency of purity

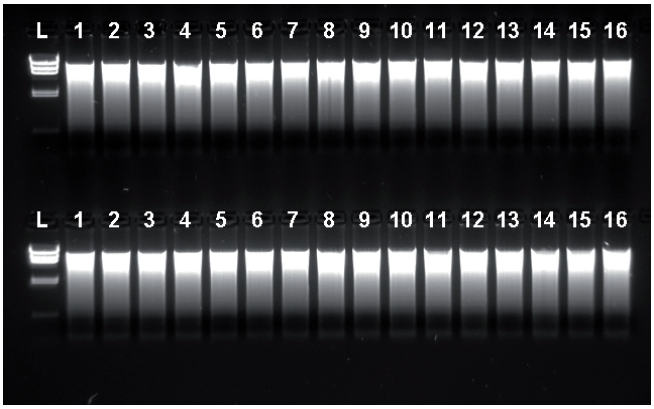

Figure 3: Agarose gel analysis of purified DNA. 15µl of randomly selected samples were loaded on a 1% (w/v) TAE agarose gel. DNA size standard: lambda/Hind III DNA.

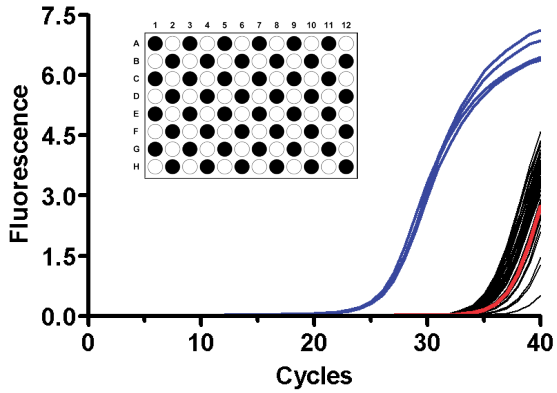

Figure 4: A) Plate set-up to test cross contamination. Black wells: Lysates. White wells: Buffer
B) Real-time qPCR-analysis (SYBR-green) of the eluates. Blue lines: DNA eluted from mouse tail end clippings. DNA was diluted 10fold for PCR. Crossing point: 22. Black lines: Eluates from buffer extract. DNA was used undiluted for PCR. Crossing Points: >36. Red line: negative control. Detection limit: 0.2ng DNA.

and yield (Fig. 3). Cross contamination testing proved that carryover to neighboring wells did not occur down to the detection limit of 0.2ng DNA (Fig. 4b). Further, an agarose gel and a melting point analysis were performed of the eluates from the buffer extraction and negative controls. The analysis showed that the products were caused by unspecific amplification by primer dimers. The Genomic STARlet could process 48 samples with the 8-well strips or 96 samples with the 96-well binding plates in less than 80min.

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 samples. Here, we demonstrate the automated isolation of genomic DNA from mouse tail end clippings. The automated and manual isolation resulted in similar yields and qualities of DNA. The use of the MN Wash Plate during vacuum processing effectively prevents ethanol contamination derived from ethanolic wash buffers..

Therefore, the automated isolation of DNA from tissues on the Genomic STARlet guarantees increased standardization for downstream genotyping applications.

MACHEREY-NAGEL

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