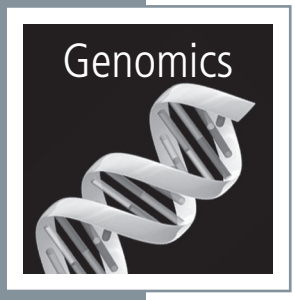

Fully Automated Genomic DNA Isolation from mouse tails on the MICROLAB® STAR

Reliability and process control are major issues for automated genomic DNA isolation from mouse tails. The new automated solution from HAMILTON on the MICROLAB® STAR liquid handling robot using NucleoSpin® technology from MACHEREY-NAGEL features 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,caused by particulate matter or debris in starting 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 MACHEREY-NAGEL gDNA Isolation from mouse tails includes:

- MICROLAB® STAR, 8 channels, with built-in robotic plate-handler (iSWAP), manual or autoload
- MICROLAB® BVS Basic Vacuum System
- MICROLAB® STAR Shaker for sample lysis on deck (Variomag® Thermoshake, H+P Labortechnik, Oberschleissheim, Germany)
- All required carriers and the complete method

Reagents

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

Protocol

Deck Layout

The deck is manually loaded with carriers containing tips, reagents, mouse tail samples, microplates and filter plates. The mouse tail samples can either be lysed manually by the user (incubation of tail clippings in lysis buffer T1 on a shaker/incubator at 56°C overnight) or on the deck of the MICROLAB® STAR using an integrated Thermoshaker.

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

Lysis buffer T1, proteinase K and 0.4cm individual mouse tail end clippings (approx. 10mg) are transferred to the lysis block. After the addition of the lysis buffer, the samples are incubated and shaken overnight at 56°C on the Variomag Thermoshake. This step can also be performed manually on suitable shaker/incubator. Following lysis incubation, binding buffer BQ1 and ethanol is added to the crude lysates and mixed well before the samples are transferred to the NucleoSpin® Tissue binding plate followed by a vacuum filtration binding step on the MICROLAB® BVS. After the binding of the DNA, the silica membranes are washed three times with two different wash buffers. Once the NucleoSpin® Tissue Binding 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.

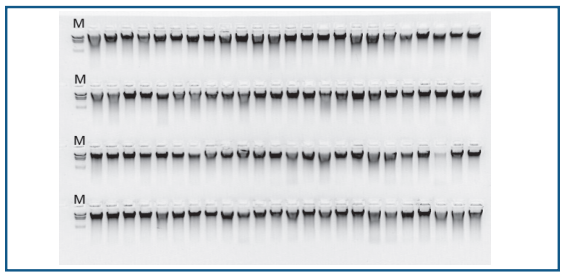

Figure 1: *Isolation of genomic DNA from individual mouse tails. Samples were lysed overnight (proteinase K treatment) at 56°C. After a shorth centrifugation step all further steps were performed on the MICROLAB® STAR. Yields of 4.9 to 12 µg (average: 8.93) with a purity OD260/280 of 1.9 were obtained. DNA was eluted with 200 µl buffer BE. 10 µl of the recovered DNA was loaded on a 1% TAE agarose gel, M: molecular weight marker.*

HAMILTON

Validation

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

In the shown experiment 96 individual mouse tail end clippings were used to isolate genomic DNA on the MICROLAB® STAR using the NucleoSpin® 96 Tissue kit and the method described above. The isolated genomic DNA was analysed on agarose gels and with absorption readers. The quality, reproducibility and usability in downstream applications 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, with an average yield of 8.93 µg per sample.

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

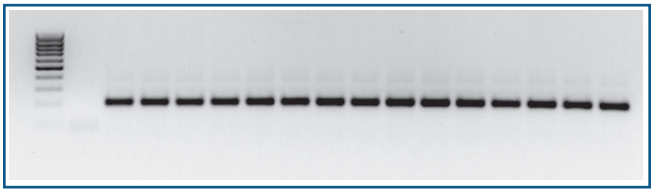

Figure 2: *PCR amplification of randomly selected purified DNA samples. 2 µl of each recovered DNA was amplified in a 35 cycles PCR reaction. The 212 bp PCR product for the* Mus musculus cytoplasmic aconitase exon *(aco I) was generated. All samples were amplified. Lane 1 contains a molecular weight marker, lane 2 a negative control.*

Usability in downstram applications

To test for usability in downstram applications like enzmatic treatments, 1µg of randomly selected samples was subjected to a restriction enzyme digest with 1 unit *Eco R* I. All samples were completely digested (Figure 3).

Throughput and Capacity

The isolation of 96 mouse tail samples with the NucleoSpin® 96 Tissue 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.

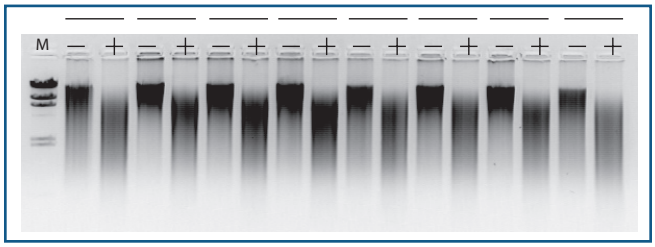

Figure 3: *Restriction analysis of randomly selected purified DNA samples. 1 µg of DNA purified with NucleoSpin® 96 Tissue on the Hamilton MICROLAB® STAR was restricted with* Eco R *I (1u) for 1h at 37°C (+). Control samples were incubated for 1 h in enzyme reaction buffer without enzyme at 37°C (-). Samples were analyzed on 0.8% TAE agarose gel. (M) λ/HindIII DNA marker. All samples incubated with enzyme were restricted. No degradation of control samples confirms high purity of purified DNA and absence of DNase activity.*

Discussion

HAMILTON and MACHEREY-NAGEL have developed a validated method for fully automated genomic DNA isolation from mouse tail clippings 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 reaction setup 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 clot detection
- Sample tracking available as an option
- Automation of additional applications like PCR preparation or DNA digestion

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