

Mole®

DNA extraction from mouse tissue

“ GeneMole® is a benchtop instrument for automated nucleic acid purification. Automation reduces the risk of injuries caused by repetitive pipetting, limits exposure to chemicals and infectious agents, and gives you more time to focus on other operations. GeneMole® can process 1-16 samples in one run and all the reagents required are available as pre-filled disposable MoleStrips™. ”



Figure 1: The mouse - a primary mammalian model system for genetic research.

Introduction

Scientists from a wide range of biomedical fields have chosen the mouse as a model because of its close genetic and physiological similarities to humans, as well as the ease with which its genome can be manipulated and analyzed (1). Many laboratories use manual purification methods to isolate genomic DNA from mouse tissue. Here the GeneMole® instrument and MoleStrips™ DNA Tissue reagents are used for automated isolation of genomic DNA from different mouse tissues.

Methods

Mouse tissue samples were stored at -80 °C prior to DNA extraction. Genomic DNA was isolated according to the protocol given in the MoleStrips™ DNA Tissue kit. The elution volume was specified as 200 µl. DNA yield and purity were determined using Perkin Elmer Lamda EZ 201 UV/Vis Spectrophotometer. Real time PCR was performed using primers targeting the β-actin gene. Data was generated on Applied Biosystems 7300 Real time PCR system and analysed with 7300 System SDS Software. Primers were from ABI.

Result

Genomic DNA was isolated from several different mouse tissues as shown in figure 2.

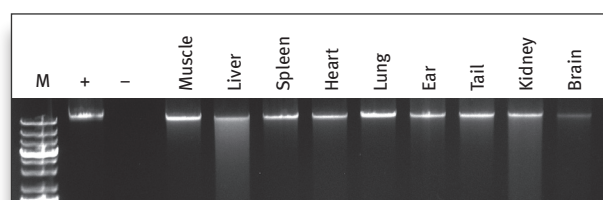


Figure 2: Agarose gel (0.8%) with 100 ng genomic DNA extracted from different mouse tissues. M: GeneRuler DNA Ladder Mix Plus (Fermentas), + : mouse genomic DNA (Novagen), - : neg. control.

The β-actin gene was successfully amplified using PCR from all the extracted genomic DNA samples. As template in the PCR reaction 3 µl of the total 200 µl eluted genomic DNA was used.

Ten-fold dilution experiments with real time PCR indicated the absence of PCR inhibitors in the isolated DNA from all the tissues tested. Figure 3 shows an example of such a real time experiment.

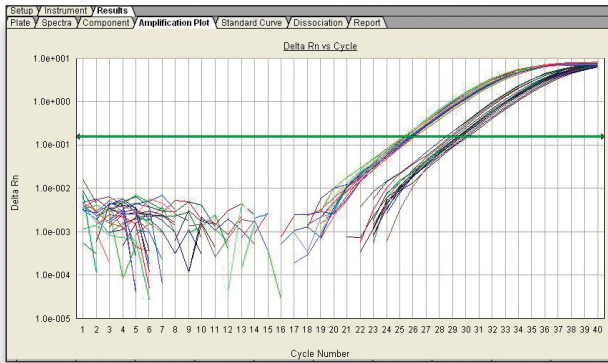


Figure 3: Logarithmic amplification plot from real time PCR. 16 genomic DNA samples isolated from muscle were diluted 10-fold. Both undiluted and diluted samples were amplified by real time PCR. The average ΔC_t value of 3.6 indicated that no PCR inhibitors were present in the sample.

All tissues are different in composition and therefore the optimal amount of starting material and the final DNA yield will vary from tissue to tissue. In Table 1 the recommended amount of starting sample as well as the expected purity and yield data for different tissue samples are listed.

Table 1: Optimal amount of starting sample and expected DNA yield for different mouse tissues.

Tissue	Starting sample [mg]	A260/A280	DNA conc. [$\mu\text{g}/\text{ml}$]	Yield [μg]
Muscle	10	1.89	23	4.6
Liver	10	1.98	92	18.4
Spleen	1	1.92	51	10.2
Heart	10	1.93	21	4.2
Lung	3	1.93	48	9.6
Ear	5	1.96	62	12.4
Tail	5	1.91	34	6.8
Kidney	10	1.91	84	16.8
Brain	10	1.95	33	6.6

The reproducibility of the system was investigated by setting up full 16 runs with muscle tissue from mouse lysates. High molecular weight DNA was consistently isolated from all 16 samples (Figure 4A), and the measured DNA yield was similar ($\sim 20 \mu\text{g}/\text{ml}$) for all samples in the run with a %CV of 3.4 (Figure 4B).

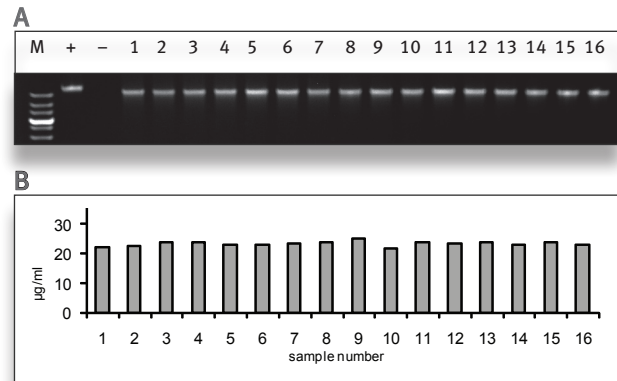


Figure 4: Genomic DNA extracted from 16 mouse muscle lysates. (A) Aliquots ($5 \mu\text{l}$) of each eluate analysed by 0.8% agarose gel electrophoresis. (B) Genomic DNA yield obtained for each of the 16 lysates, (%CV = 3.4).

Conclusion

The GeneMole® instrument is ideal for automated DNA extraction from mouse tissues using MoleStrips™ DNA Tissue kit.

As expected, the yield of genomic DNA varies from tissue to tissue (see Table 1).

The results obtained are reproducible and the extracted genomic DNA is of high quality, free from PCR inhibitors and ready to be used in sensitive downstream applications.

Reference

(1) Background on Mouse as a Model Organism, National Human Genome Research Institute (<http://www.genome.gov/10005834>).

“Simplify When Possible”

Ordering Information

Product

GeneMole®

MoleStrips™ DNA Tissue

MoleStrips™ DNA Tissue Convenience Kit¹

Content

Instrument

64 Reagent Strips

32 Reagent Strips

Product Number

MG10-000-000

MGK20-101-102

MGK20-101-101

¹ The convenience kit contains 32 Reagent Strips and all the required tips, tubes and caps.