Mole®

RNA extraction from mouse tissues

“GeneMole® is a robust benchtop instrument that provides automated extraction of high quality nucleic acid. With reduced hands-on time, automation allows users time to focus on other operations as well as reduced exposure to harmful agents. GeneMole® can process 1-8 RNA samples in one run and all the reagents required are available as pre-filled disposable MoleStrips™.”

Figure 1
RNA extraction from various soft tissue can be automated on the GeneMole® system.

Introduction

The GeneMole® instrument and the MoleStrips™ RNA Tissue Kit can be used for automated isolation of RNA from non-fibrous animal tissues. Fresh animal tissue, tissue stabilized in RNA*later*™ or tissue frozen directly on dry ice or liquid nitrogen can be used for RNA extraction. The isolation procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. After magnetic separation and removal of the supernatant, the paramagnetic beads are washed several times with different wash buffers to remove contaminants and salts. Finally, highly purified RNA is eluted and is ready for use in downstream applications.

Below, the GeneMole® instrument and MoleStrips™ Total RNA Basic Kit have been used for automated isolation of RNA from various soft mouse tissues.

Woking with RNA

The condition of the starting material is crucial for the quality of the extracted RNA. Endogenous RNases must be inactivated immediately upon tissue harvesting and cell death to prevent RNA degradation. There are different methods to accomplish this: 1) Directly disrupt and homogenize the sample in the presence of RNase-inhibiting or denaturing reagents, 2) “Flash freeze” sample in liquid nitrogen, or 3) Quicly place sample in RNA*later*™ stabilizing solution (animal tissues only). Autoclaving tips, tubes and solutions is not sufficient to inactivate RNases. To ensure intact, high quality RNA, it is essential to reduce exposure to environmental RNases. All surfaces, including pipettors, benchtops, glassware, and gel equipment should be decontaminated with a surface decontamination solution. When working with purified RNA, ensure samples remain on ice during downstream applications.

Methods

Typically 1-8 samples can be processed in one run, using anything between 1-10 mg of tissue. Disrupt the tissue in 375 µl Mole Lysis buffer, containing RNase-inhibiting and denaturating reagents.
Disruption of tissue sample by homogenization need to be optimized for each specific tissue sample. Homogenization is often performed using a mechanical shearer, a “bead beater” (*e.g.* Precellys 24, Bertin Technologies) or grinding by mortar and pestle. Centrifuge the lysate for 1 minute at 10,000 rpm, and then transfer 350 µl of the lysate to GeneMole® sample tubes. Add 5 µl glycogen solution to each sample and place the tubes in the GeneMole® worktray. We recommend to use 5 µl glycogen solution for each 10 mg tissue sample.

Mouse Tissue	Recommended input [mg]	Conc. ng/µl no DNase treatment	Conc. ng/µl DNase treatment	Ratio 260/280	Ratio 260/230
Liver	10	250-450	60-300	>1,9	>1,8
Brain*	10	60-200	20-65	>2,0	2,0
Kidney	10	180-280	180-280	2,0	>1,8
Spleen	2	300-350	50-110	>1,9	>1,8
Lung	10	40-70	25-50	>2,0	>1,7

Table 1
Optimal amount of starting material and the expected RNA yields for various mouse tissues when eluting in 100 µl elution buffer. RNA integrity number (RIN) of listed tissues were all above 8 and for both +/- DNase treatment the 260/280 and 260/230 ratios were equivalent.
* To improve performance and yield a pre-treatment is required.
More information visit: www.molecookbook.com

Results

All tissues are different in composition and therefore the optimal amount of starting material and final yield will vary, accoring to the biological sample chosen and the metabolism of the cells. Table 1 shows the recommended amount of starting material and the typical yields for various mouse tissues.

Using intact RNA is a key element for successful microarray or RT-PCR analysis. RNA extracted with Gene**Mole**® instrument typically has RNA integrity number ranging from 8-10 and thus it is considered suitable for most gene expression studies.

Currently no available purification method can guarantee that the RNA is completely free of DNA. As PCR can detect even a single molecule of DNA, RNA samples should be digested with DNase I before RT -PCR to avoid false positives and high background. In order to be flexible, two protocols have been optimized for the Gene**Mole**®, with one protocol including automated DNase treatment within the isolation procedure. Figure 2 visualises the results of RNA extracted from both protocols, analyzed by agarose gel electrophoresis.

The success of the DNase treatment was verified in a RT/-RT real-time PCR experiment. Additionally, tests on purity and stability have been performed, showing extracted RNA samples free of contaminants and of high purity.

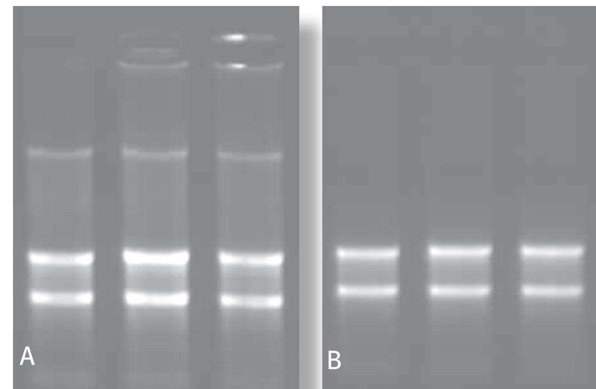

Figure 2
1% agarose gel of RNA eluates extracted from 3 mouse liver samples.
A) Results using the protocol without DNase treatment.
B) Results using the protocol including DNase treatment.

Comments

The quality of the starting material must always be considered when evaluating quantity and quality of the extracted RNA.

To prevent interference by DNA, we recommend designing primers that anneal at intron splice junctions as this will prevent genomic DNA being amplified.

"Simplify When Possible"

Ordering Information

Product	Content	Prod. No.
Gene**Mole**®	Instrument	MG10-000
MoleStrips™ RNA Tissue	64 Reagent Strips	MG11-105