

Mole®

Nucleic acid extraction from zebrafish samples

“ GeneMole® is a benchtop instrument that provides automated nucleic acid purification. 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 1-16 samples in one run and all the reagents required are available as pre-filled disposable MoleStrips™. ”



Figure 1:
Zebrafish embryo, 48 hfp.
The Zebrafish is an important genetic model organism used to study vertebrate development and diseases.

Introduction

Zebrafish (*Danio Rerio*) is a small, freshwater fish, native to the streams of the Himalayan region. The zebrafish is widely used among scientists as a model organism because it appears to combine the best features of other models. The zebrafish embryo is large, robust, transparent, and develops externally to the mother, allowing for easy manipulation and observation. Zebrafish has generation intervals similar to mice (2- 3 months), but produce more offspring in a shorter time.

The zebrafish is a popular genetic model also because its genome is fully sequenced and well annotated. There are extensive similarities between the zebrafish and the human genome, and a number of zebrafish mutants have been produced that are good models of human diseases. Among them are models of Alzheimer's disease, congenital heart disease and cancer.

The GeneMole® instrument together with MoleStrips™ can be used for automated extraction of nucleic acids from zebrafish samples. 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 to remove contaminants and salts. Finally, highly purified nucleic acids are ready for use in downstream applications.

Methods

GeneMole® is constructed to handle an input of 1-20 mg of tissue or 1-2 million cultured cells. Both fresh and frozen samples can be used for extraction. DNA extraction from muscle and fins were performed according to the protocols given in the MoleStrips™ DNA Tissue kit. The samples were incubated overnight at 56°C with agitation. After incubation the samples were briefly centrifuged in order to get rid of undissolved bone components prior to loading on the GeneMole® instrument.

Embryo, ovary, brain- and liver tissues were frozen in liquid nitrogen and stored at -80°C prior to RNA extraction. RNA was extracted according to the protocols given in the MoleStrips™ RNA Cell kit and MoleStrips™ RNA Tissue kit.

Table 1: Recommended input and expected yield for different zebrafish samples tested on the GeneMole® instrument. RNA from zebrafish embryo was tested at different hours post fertilization (hfp).

Application	Kit	Input	Elution volume [µl]	Yield [µg]	Concentration [µg/ml]	A260/A280
DNA muscle	DNA Tissue	~10 mg	100	4.7	47	1.94
DNA fins	DNA Tissue	~10 mg	100	5.1	51	2.03
RNA brain	RNA Tissue	~6 mg	100	3.8	38	2.1-2.2
RNA ovary	RNA Tissue	~13 mg	100	40	400	2.0-2.1
RNA liver	RNA Tissue	~7,5 mg	100	76	762	2.1
RNA embryo, 4-5 hfp	RNA Cell	50 embryos	100	7-12	70-120	2.1
RNA embryo, 24 hfp	RNA Cell	30 embryos	100	7-8	70-80	2.1
RNA embryo, 48 hfp	RNA Cell	20 embryos	100	7-7.5	70-75	2.1

Results

The optimal input of cells and the final yield of nucleic acids, will vary according to the size and composition of the cells. For RNA especially, yield will also vary as a result of the biological metabolism of the cells. Table 1 shows the recommended amount of starting material and the yields for different zebrafish tissues/cells on the GeneMole® instrument. Yield and purity were determined using the Perkin Elmer Lamda EZ 201 UV/Vis Spectrophotometer or NanoDrop™ 1000 Spectrophotometer (Thermo Scientific). The assessment of RNA integrity was performed using the Bioanalyzer (Agilent Technologies) and all RNA integrity numbers (RIN) were above 9.

As PCR can detect even a single DNA molecule, to avoid false positives and high background, RNA samples should be digested with DNase prior to RT-PCR reactions. To prevent interference by DNA, we recommend, designing primers that anneal at intron splice junctions as this will prevent genomic DNA being amplified.

In order to be flexible, two protocols have been optimized for the GeneMole® instrument, with one protocol including automated DNase treatment within the isolation procedure. The success of DNase treatment has been verified with RT/-RT real time PCR experiments, showing extracted RNA free of contaminants and of high purity and quality.

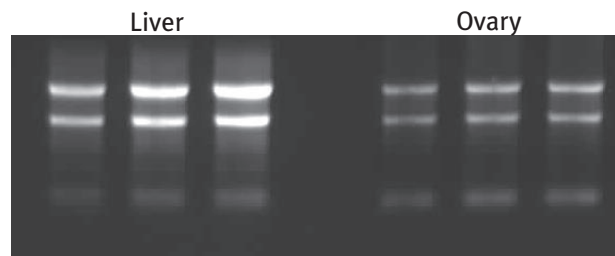


Figure 2: 1% Agarose gel analysis of 2.5µl RNA eluates extracted from liver and ovary tissue.

Conclusion

The GeneMole® instrument is ideal for automated DNA and RNA extraction. It strongly reduces hands on time and provides nucleic acids of high yields and quality.

Acknowledgment

Zebrafish samples were kindly provided by Peter Alestrøm at the Norwegian Veterinary School, Department of Basic Sciences and Aquatic Medicine.

Reference

http://genome.wellcome.ac.uk/doc_wtdo2o8o6.html

“Simplify When Possible”

Ordering Information

Product	Content	Product Number
GeneMole®	Instrument	MGI10-000-000
MoleStrips™ DNA Tissue	64 Reagent Strips	MGK20-101-102
MoleStrips™ DNA Tissue Convenience kit ¹	32 Reagent Strips	MGK20-101-101
MoleStrips™ RNA Cell	64 Reagent Strips	MGK30-102-102
MoleStrips™ RNA Cell Convenience kit ¹	32 Reagent Strips	MGK30-102-101

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