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Evaluation of different RNA extraction methods for small quantities of tissue from the marine flatworm *Macrostomum lignano*

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Introduction

Because growing large amounts of animals is time consuming and labour intensive, it is very useful to search for an effective method for RNA isolation that requires as small amounts of animals as possible. But isolating RNA from small animals or quantities can be a delicate procedure. Highly sensitive techniques for transciptome analysis, such as real-time PCR, micro arrays and others currently used in functional genomics require a high integrity and quality of the RNA, as well as reproducibility between replicates of the same tissue. The new model-organism, *Macrostomum lignano* is a small marine flatworm that has an average weight of 350 µg and is 1.2 mm long. Isolation of RNA from small quantities of tissue still needs a lot op optimalisation. Because culturing *large amounts of Macrostomum lignano* is labour intensive our goal was to isolate RNA from small quantities of sample material.

Materials and Methods

Different lysing methods with the RNeasy micro kit (Qiagen)

1. Mixer mill (Retsch GmbH)
   Different amplitudes and durations of mixing were tested with glass and metal balls.
2. Sonication (Sonomatic 300, Reinigungsgerät)
   RNA later (Qiagen) was added to the animals The samples were sonicated for 10 minutes.
3. Chemical lysation
   25 µl SDS buffer, 2,5 µl proteinase K, 1 µl betamercapto-ethanol and RNA later
   were added to the animals. This mixture was heated for 30 minutes at 56°C.

RNA extraction

1. RNeasy micro kit, Qiagen: absorption on silicon membrane, 25 animals
2. RNeasy mini kit Qiagen: absorption on silicon membrane, 50 animals
3. QuickPick™ mRNA kit (Isogen Life Sceinces), absorption on metal particles, 30 animals
4. Trizol®reagent, (Invitrogen) 500 animals

RNA concentrations and purity was assessed using a nanodrop ® -ND 1000 UV-Vis Spectrophotometer. RNA integrity of the extracted RNA molecules was assessed using an Agilent 2100 Bioanalyzer with the RNA 6000 Nano labChip® kit.

Results

Lysation

* Mixing, followed by sonication yield was the highest amount of RNA. Chemical lysing was the least efficient method (see table 1).
* Using glass balls resulted into higher RNA yield (see table 1).
* Only mixing maintained the integrity of the 18S and 28S RNA (see fig. 2).

RNA Isolation

* RNA yield was highest with RNeasy minikit, Qiagen (see table 2)
* Using metal particles gave inconsistent result
* Trizol extraction had very little yield
* RNA integrity was not maintained with QuickPick™ mRNA, Invitrogen and trizol® reagent, Invitrogen (see fig. 4)

New Model organism

Macrostomum lignano (Ladurner et al., 2005) is a member of the Macrostomorpha, the basal-most subtaxon of the Platyhelminthes–Rhabditophora. This new species has been already the subject of several developmental/evolutionary studies.

These animals are easy to culture, and with their small size (1,2 mm), (only about 25 000 cells) constituting the major bilaterian organ systems.

Flatworms (Plathyhelminthes) are famous for their ability to regenerate. These animals are therefore intensively studied in stem cell research. But they also contribute effectively in toxicity research, reproduction and many other widespread branches of scientific research.

Fig. 1. Adult indivual of *M. lignano*

Table 1: Methods of lysation and RNA yield

Lysing Method	RNA yield (ng/µl)
Mixer, glass balls	84,13±9,08
Mixer, steal balls	72,31±13,05
Sonication	44,71±18,96
Chemical lysing	27,54±18,69

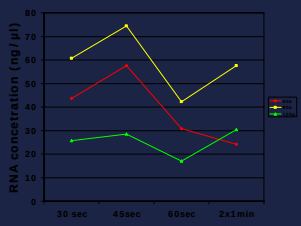

Fig. 3. RNA mixing efficiently with glass balls.

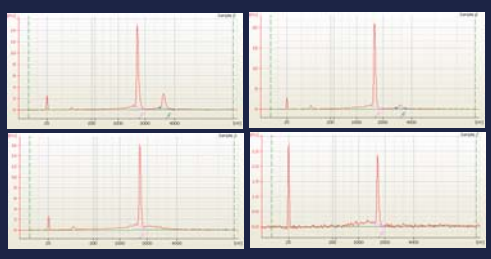

Figure 2. RNA integrity assessment by bioanalyser analysis. Electropheregrams of differenent lysing methods are shown, a. mixing with glass balls, b. mixing with steal balls, c. sonication, d. chemical lysation

Table 2: Methods of extraction and RNA yield

Method	RNA yield (ng/µl)
Silcon membranes	
1. RNAesy microkit (Qiagen©)	84,13±9,08
2. RNAesy minikit (Qiagen©)	68,50±9,33
QuickPick™ mRNA, Invitrogen	40,67±35,94
Trizol®reagent	10,4±3,79

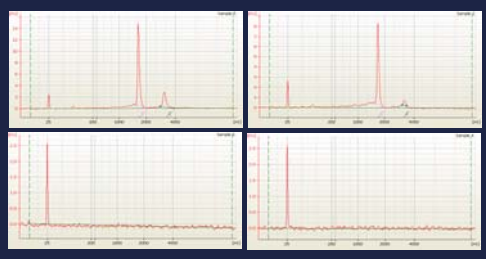

Figure 4. Electropheregrams of differenent extraction methods are shown, a. RNeasy microkit, qiagen, b. RNeasy minikit, qiagen, c. Quickpick mRNA ™, Invitrogen d. Trizol®reagent

Discussion and conclusion

Lysation of tissues is a very delicate step during RNA isolation, because as little material as possible should get spilled. Therefore, use of a mixer mill or sonication device seems to be the adequate method, as the eppendorf tubes stay closed, preventing loss of material. Our study shows that for our test organisms, the Mixer mill method is superior to sonication, yielding higher amounts of RNA. Chemical lysation appeared to be the least efficient method.

From the kits available commercially for RNA extraction for small quantities of tissue, the silicone membrane based kits appeared to be the most efficient in our case. Extractions were successful even when using as few as 25 animals. By contrast, extraction using Trizol®reagent did not yield sufficient amounts of RNA, even when using 500 animals or more were used.

Based on these results, we can conclude that, for *M. lignano*, silicon membrane extraction (RNeasy microkit, Qiagen) with the Mixer mill, preferably with glass balls, will result in the highest yield and the least degraded RNA.