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Automatization and standardization of RNA purification for microarray experiments

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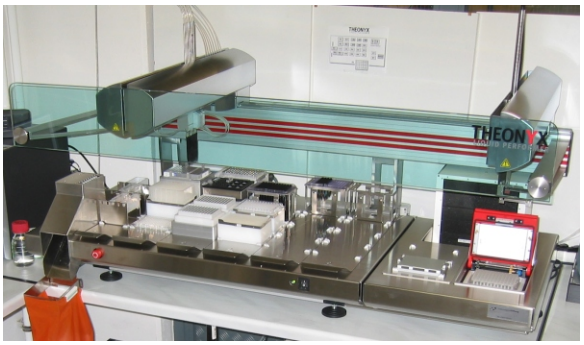
Introduction

Obtaining high quality, intact RNA is the first and often the most critical step in performing many fundamental biology experiments including transcript profiling.
However, successful RNA isolation requires attention to handling and fast processing of the tissue or the cells. Additionally, impurities in RNA preparation can have an adverse effect both on labeling efficiency and the stability of the fluorescent labels. Therefore, a major point will be the standardization of RNA purification and its conversion into a fluorescent labeled cDNA using a liquid performer.
Comparing several methods for RNA isolation we have found that the THEONYX™ Liquid Performer in combination with the Promega SV 96 Total RNA Isolation System™ gives consistently high quality RNA from cell culture as well as from tissue samples.



Material and Methods

THEONYX™ Liquid Performer

The Robotic liquid handling workstation uses two robotic arms to affect automated liquid handling (pipetting) and robotic transportation of microplates.
The Pipetting arm carries eight tips. Each has an individual level detector and Y- and Z-motor. All tips
are connected to a 2-port valve of a dedicated precision mini-tooth wheel pump, located in the robotic arm, which controls the aspiration and dispension functions.
The integrated vacuum station for filter plates enables the simultaneoulsly RNA purification of 96 probes.

Promega

SV 96 Total RNA Isolation System

The RNA purification kit of Promega is a ready-to-use kit integrated all plates and chemicals except ethanol for a complete 96-well-binding plate.
The Promega SV 96 Total RNA Isolation System was used to purify the RNA from *E. coli* and *S. cerevisiae*.
For cell lyses were used the enzymes lysozym for bacteria and lyticase for yeast.

Results

The quality and quantity of the purified RNA was characterized using the following methods:

Yield and Purity

By measuring the ratio of the optical density (OD), OD260/OD280, it is possible to not only measure the concentration of the RNA, but also to get a rough estimate of the amount of contamination from proteins (or phenol) that were not removed during the purification procedure
The total yield of RNA from $5 \cdot 10^9$ *E. coli* cells was up to 120 µg with a good ratio.

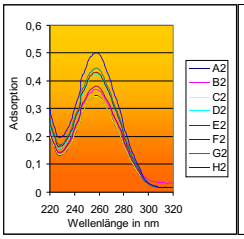


Fig. 1: Wavelength scan of purified RNA

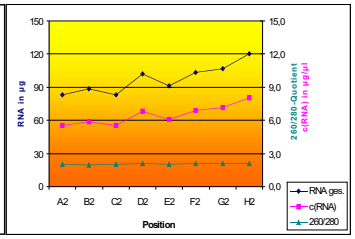


Fig. 2: Yield of purified RNA

Detection of RNA in Agarose Gels

10 µg denatured RNA samples were electrophoresed on a 1.0% agarose gel containing formaldehyde and visualized with ethidium bromide staining.

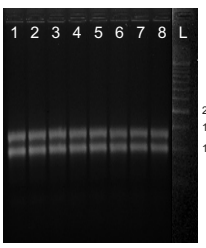


Fig. 3. Agarose Gel Analysis of Total RNA

Agilent 2100 bioanalyzer

The Agilent 2100 bioanalyzer and the RNA Nano 6000 LabChip® kit provide a simple, rapid alternative for the characterization of RNA samples. The 25-min-protocol allows the determination of concentration and purity/integrity of 12 RNA sample.

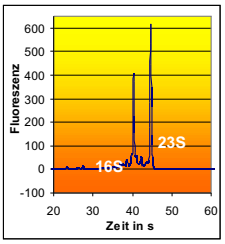


Fig. 4: Analysis of high quality total RNA with the 16S and 23S subunit as two distinct bands

Dot Blot Analysis of DIG-labeled cDNA

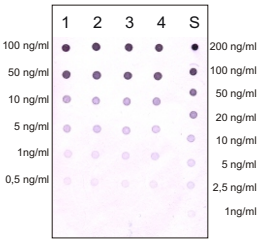


Fig. 5: Dot Blot Analysis of DIG-labeled cDNA

Complex cDNA probes were generated from total RNA using AMV Reverse Transcriptase that incorporates DIG-11-dUTP into new synthesized cDNA. The estimation of yield is performed by side comparison of the DIG labeled probe with a DIG labeled control DNA. Dilution series of both are prepared and spotted on a piece of membrane. Subsequently, the membrane is chemiluminescently detected and direct comparison of the intensities of sample probes and control allows the estimation of labeling yield.

Summary

Using THEONYX™ Liquid Performer to fully automate RNA purification decreases the risk of RNA degradation and cross contamination. The greatest benefit of the THEONYX™ System is the standardization of methods for very fast purification of RNA, with high quality and high throughput.