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TheOnyx

Whole RNA Isolation, automated with TheOnyx

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Introduction

Gene expression studies are a universally established tool in academic & drug discovery research. As a result, core facilities and other medium to high throughput microarray laboratories may experience bottlenecks in the upstream isolation of whole RNA from a wide range of human and animal cells or tissues. Manual isolation of RNA from different sources is time consuming, monotonous and liable also to human error – results are only as accurate and reproducible as the sample preparation and reaction set-up methods employed.

We describe a fully automated procedure for the isolation of whole RNA from mammalian tissue with TheOnyx, using the NucleoSpin® Multi-96 RNA kit from Macherey-Nagel. The kit is designed for rapid, simultaneous preparation of highly pure total RNA in a format suitable for complete robotic automation. Following tissue homogenization, all steps of the kit protocol are performed on TheOnyx with no user intervention throughout.

Principle of RNA isolation

The method is based on reversible adsorption of RNA to a silica membrane. The tissue is first homogenized in a lysis buffer containing a high concentration of chaotrophic salts.

This lysis buffer inactivates RNases and provides suitable conditions for the RNA to bind the silica membrane. Contaminating DNA is removed by DNase I digestion directly onto the silica membrane during the purification procedure.

After several washing steps the pure RNA is finally eluted with RNase-free water under low ionic strength conditions.

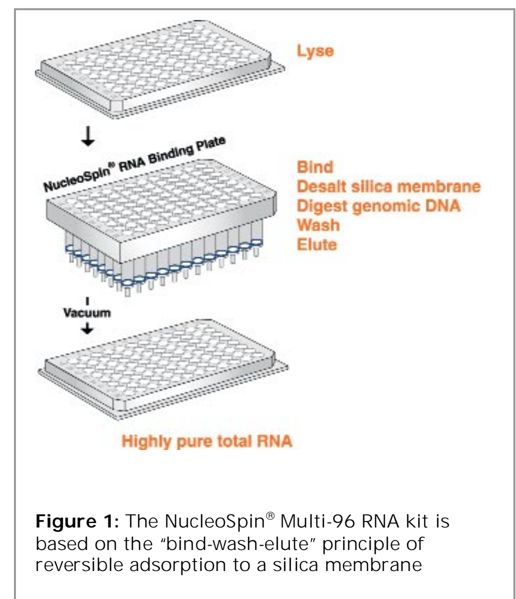

Figure 1: The NucleoSpin® Multi-96 RNA kit is based on the "bind-wash-elute" principle of reversible adsorption to a silica membrane

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Smart tip logic drives TheOnyx

TheOnyx incorporates the most flexible tip handling logic available on any robotic liquid handling workstation. Disposable tips in a range of sizes from 10 µl up to 1 ml, with and without filter barriers, are placed on the platform according to the specific requirements of the user. This capability is essential where a wide range of liquid volumes need to be handled in the same application script, for example total RNA isolation (medium to high volumes) and downstream RT-PCR reaction set-up (low to medium volumes). When writing a script in TheOnyx's RoboManager software, the user selects any tip for any pipetting step entirely on demand. The software tracks the number of tips taken from each tip rack as it goes, and automatically selects the next available tip from each partly-used rack as required. In addition, the ability to stack tip racks one on top of another greatly increases the tip storage capacity on the platform. The gripper tool moves empty tip racks to free positions on the platform, which themselves are pre-determined automatically by the RoboManager software.

Homogenisation of samples

Customer samples (mouse brain & liver tissue samples were kindly provided by Pekka Kallunki, Lundbeck A/S, Copenhagen) are transferred into tubes for homogenisation and weighted. Lysis buffer and homogenising beads are added to each sample, and the tubes placed in the tissue homogenizer (FastPrep® 120A, Q.biogene Inc.) for 20 seconds. After homogenisation, samples are incubated on crushed ice for 5 minutes, and then centrifuged for 5 minutes at 14,000 rpm. Supernatants are transferred to fresh 1.5 ml Eppendorf tubes, and the samples centrifuged for a further 5 minutes. The supernatants (130 µl) are once more transferred to fresh tubes and immediately stored on crushed ice or frozen at –80°C. Samples are transferred to a deep well microplate immediately prior to processing on the robot.

Method

The robot worktable and components are first cleaned thoroughly with RNase® Zap (Ambion Inc.). Cleared homogenates in a deep well plate are placed on the robot together with all other necessary components including disposable tips (the protocol uses both 175 µl & 1,000 µl disposable tips), Macherey-Nagel NucleoSpin® Multi-96 RNA kit filter plate, collection plate & buffers. The purification procedure is completed without any further manual intervention.

- 130 µl washing buffer RA3 is added to each sample & mixed
- 265 µl is transferred from each sample well in the deep well plate to the corresponding well of the filter plate, which is positioned on the vacuum chamber
- Vacuum pressure is applied automatically from the RoboManager software (300 mbar, 60 seconds)

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- 500 µl desalting buffer RA3 is distributed from the reagent rack to each well of the filter plate
- Vacuum pressure is applied (300 mbar, 180 seconds)
- 60 µl DNase solution is distributed from the reagent rack to each well of the filter plate
- Samples are incubated for 15 minutes at room temperature
- Samples are sequentially washed with 500 µl buffers RA2, RA3 & RA4. Vacuum pressure is applied (400 mbar, 60 seconds) following the addition of each wash buffer
- The plate gripper moves the filter plate to a so-called "touch position" **(Figure 2).** The absorbent material of the touch position removes any remaining droplets from the tips of the filter columns
- The plate is returned to the vacuum chamber, and vacuum applied for a further 20 minutes at 500 mbar to dry the filter membrane completely
- Now the collection plate is positioned inside the vacuum chamber with the filter plate on top. All movements of the plates and the vacuum chamber collar are made automatically with the gripper tool.
- 120 µl elution buffer is distributed to each well of the filter plate
- Samples are incubated for 15 minutes at room temperature
- Vacuum pressure is applied (500 mbar, 2 minutes) and the samples eluted into the collection plate
- The collection plate containing the pure total RNA samples is moved inside the cooled stacker for safe storage **(Figure 3).**

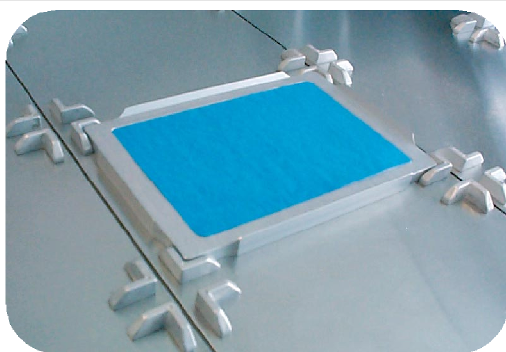

Figure 2: Lowering the filter plate onto the absorbent touch position removes residual droplets of wash buffer, essential in preventing carry-over of ethanol in the sample

Figure 3: TheOnyx's temperature controlled stacker module keeps sample plates cooled at 4°C and shielded from light. The temperature can be adjusted up to 50°C as required for other applications.

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Results

Figures 4 & 5 illustrate whole RNA samples purified according to the methods described.
In **Figure 4** (1.3% formaldehyde gel stained with ethidium bromide), the samples in lane 1 – 4 were isolated from rat brain and lanes 6 – 9 from rat liver. Lane 5 is RNA marker. Approximately 13 mg of starting tissue was used for each sample, from which 0.3 - 0.4 µg per mg of whole RNA was yielded. Measured A260/280 ratios were 1.8 – 2.0 for all purified samples. There was no detectable genomic DNA contamination. The total time needed for purification of 96 samples is approximately 1 hour.

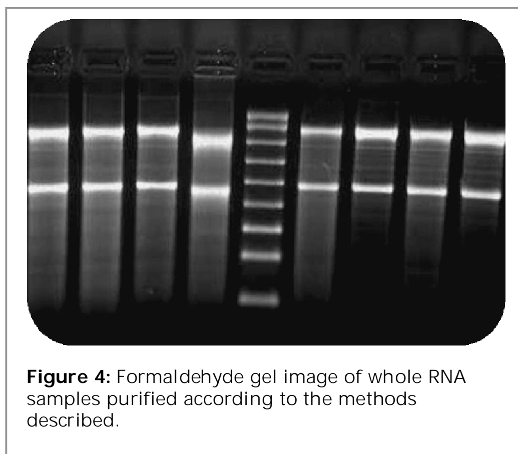

Figure 4: Formaldehyde gel image of whole RNA samples purified according to the methods described.

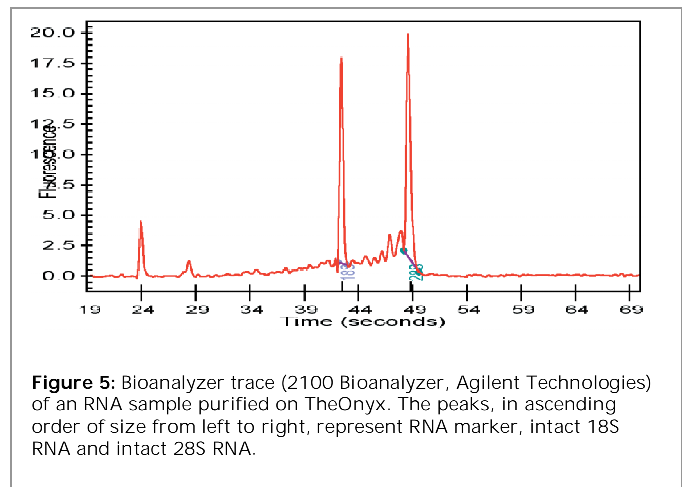

Figure 5: Bioanalyzer trace (2100 Bioanalyzer, Agilent Technologies) of an RNA sample purified on TheOnyx. The peaks, in ascending order of size from left to right, represent RNA marker, intact 18S RNA and intact 28S RNA.

Conclusions

With an established kit such as NucleoSpin® Multi-96 RNA, TheOnyx generates high yields of intact whole RNA on a medium to high throughput scale. The eluted samples are of high purity and ready to use in downstream applications including RNA amplification and quantitative real-time PCR. We compared RNA isolated on the robot with NucleoSpin® Multi-96 RNA with the Trizol® method. Results obtained following array hybridization compared favourably against the manual method. Other RNA isolation kits (e.g. Qiagen RNeasy) have been automated also on TheOnyx.

The "plug & play" open platform design of TheOnyx makes it easily configurable with alternative modules for additional applications, including downstream microarray sample preparation and/or set-up of RT PCR reactions. The user may configure the system for some or all of the steps in these processes as desired.

Acknowledgement

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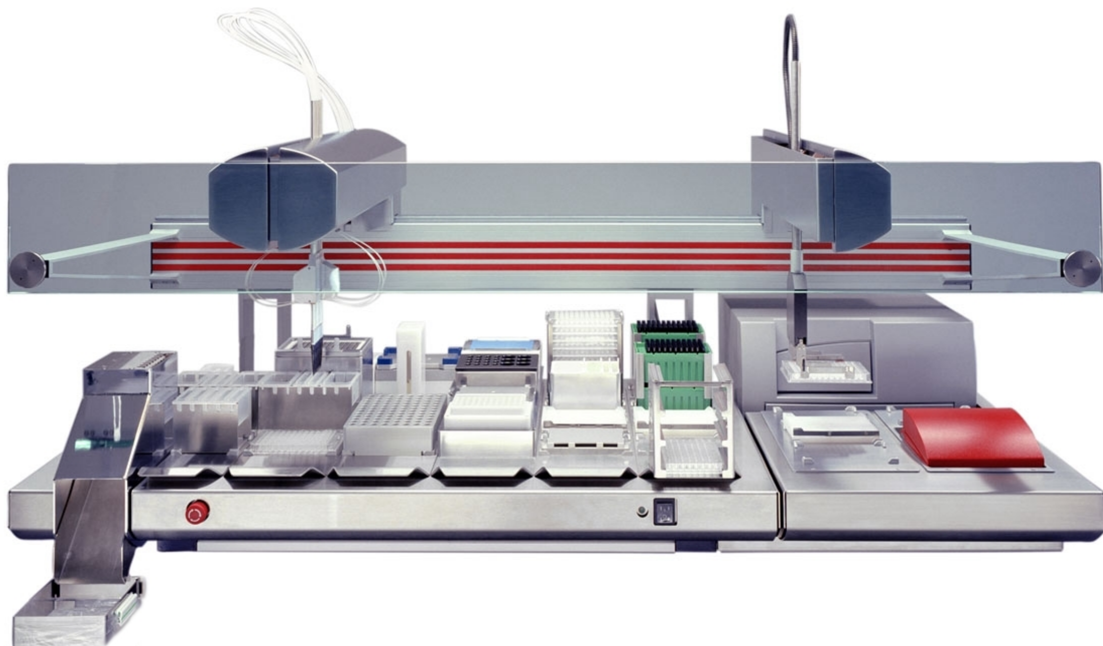

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