Automation of Micro RNA and Total RNA Purification from Tissues Using the Agencourt RNAdvance Tissue Kits and Biomek Span-8 Liquid Handler

Summary

Micro RNAs are small, naturally occurring non-coding ribonucleic acids with sizes between 18 and 40 nucleotides (nts) that have been demonstrated to play a significant role in the regulation of gene expression. As a result, interest in smaller RNA species such as miRNA has increased. This application note describes the purification of miRNA and total RNA from fresh-frozen tissue samples using the Beckman Coulter SPRI (Solid Phase Reverse Immobilization) magnetic bead-based chemistry and the Biomek automated extraction method. The RNAdvance miRNA Tissue 96 demonstrated method enables automated purification of total RNA, including miRNA and other small RNAs, from 1 to 96 samples on a Biomek Span-8 Workstation. Total RNA and miRNA can be purified from very small amounts of animal tissue as starting materials. The Biomek automated SPRI method is an easy, high-yielding and robust nucleic acid purification process that does not require centrifugation and vacuum filtration steps. Purified nucleic acids are easily eluted from the magnetic beads under aqueous conditions, which provide maximum flexibility for downstream applications. The data shows that the samples extracted using the Biomek gave comparable RNA yield, miRNA and messenger RNA gene expression as compared to samples extracted manually.

Materials and Methods

Rat liver tissue was homogenized using a Precellys 24 (Bertin) homogenizer. Eighty to 100 mg of tissue was homogenized in a CK28-7 mL tube (Bertin Technologies, KT03961-1-302.7) containing 1 mL of RNAdvance Tissue lysis buffer (Beckman Coulter Life Sciences, A32646) and anti-foaming Dx Reagent (Qiagen, 19088). Tissue was homogenized at 6,500 RPM for 20 seconds at the first cycle and 6,000 RPM for 20 seconds at the second cycle. Lysate was adjusted to 10 mg per 400 µL lysis buffer and then digested with proteinase K in a 96-Well Riplate®-2.2 mL (Ritter Medical, 43001-0020) at 37°C for 25 minutes. RNA was extracted according to the instructions for the RNAdvance Tissue miRNA protocol (AAG-230A07.14-A) using the Agencourt RNAdvance Tissue miRNA 96 (A35555 with miRNA) demonstrated method (Beckman Coulter Life Sciences). Purified RNA was eluted with 40 µL of nuclease-free water in a Hard-Shell Thin-Wall 96-Well Skirted PCR Plate (BioRad, HSP-9611). Eluted RNA concentration and purity were measured with a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific). The RNA purity was determined by the OD260/OD280 and OD260/OD230 ratios. One µL of the diluted RNA sample was analyzed on an Agilent RNA 6000 Pico chip (Agilent Technologies, 5067-1513) using the 2100 Bioanalyzer (Agilent Technologies) to determine RNA integrity. Let-7c miRNA expression was determined by a TaqMan® micro RNA assay (Life Technologies 4427975, assay ID000379). B2M cDNA was synthesized using a reverse primer (CCT GGG CTT TCA TCC TAA CA). PCR products were amplified using a primer probe mix cocktail (Forward primer TGA TCT TTC TGG TGC TTG TCT C, Reverse primer TAG CAG TTG AGG AAG TTG GG, probe CGG TGG ATG GCG AGA GTA CAC TTG). 50 ng of total RNA was used for the reverse transcription reaction using the TaqMan® micro RNA Reverse Transcription kit for let-7c and B2M

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gene expression (Life Technologies, 4366596) and 1.33 µL of cDNA was used per PCR reaction in triplicate using TaqMan® Universal Master Mix II (Life Technologies, 4440038). The detail of the RT and PCR setup was described in IB-17265A[1]. For consumables and tools used for a Biomek NX^P Span-8 automated workstation, see Table 1.

Table 1. Biomek NX^P Configuration (Tools and Consumables).

Type	Quantity	Description	Beckman Coulter Part No.
Devices	1	Orbital Shaker	379448
	1	Span-8 Passive Wash	719654
ALPS	1	Biomek NX^P Span-8 4x3 ALP Kit	989839
Magnet Plate	1	Agencourt SPRIPlate 96R—Ring Super Magnet Plate	A32782
Reservoirs	1	Reservoir Frame	372795
	1	Half Reservoir	534681
	2	Full Reservoir	372784
	2	Quarter Reservoir	372790
Consumables	8	Biomek AP96 P1000 Tip Boxes	B01123
	1	96-Well Riplate—2.2 mL	See Materials and Methods
	1	Hard-Shell Thin-Wall 96-Well Skirted PCR Plate	See Materials and Methods

Results and Discussion

Summary of RNA Yields and Purity from 96 Samples Using the Biomek RNAdvance miRNA Tissue 96 Extraction Method

An average of 10 mg of liver tissue from 96 replicates was used to evaluate RNA yield and purity using the automated RNAdvance Tissue extraction method for miRNA (Beckman Coulter Life Sciences). The RNA was eluted in 40 µL of nuclease-free water. The average concentration from 96 samples was at 1,066 ng/µL with CV % of 8.6. The calculated average yield for 10 mg of tissue was at 42.6 µg (Table 2). The OD260/OD280 ratio for 96 samples ranged from 2.04 to 2.15 with an average ratio of 2.1. The OD260/OD230 ratio for 96 samples ranged from 1.80 to 2.2 with an average ratio of 2.1 (Table 2). Figure 2 shows the examples of RNA profiling and gel view.

Table 2. Average Yield and Purity From a Total of 96 Replicate Liver Samples.

Average Conc. per 10 mg (ng/µL) ±CV%	Average Yield per 10 mg (µg)	Average OD260/OD280 Ratio	Average OD260/OD230 Ratio
1,066 ±8.6%	42.6	2.1	2.1

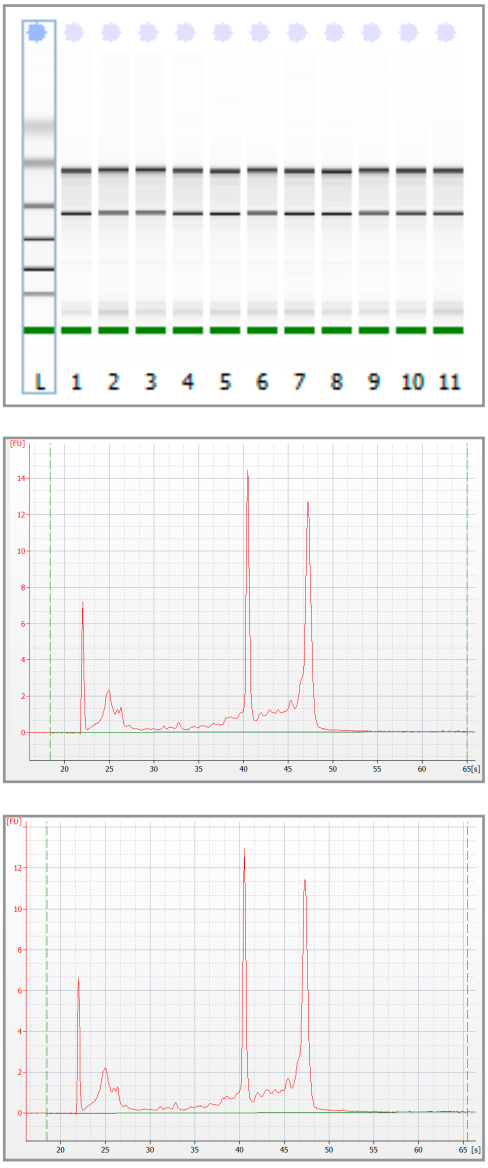

Fig. 1. Example of total RNA profiling on gel view and electropherograms. 1:1,000 dilution of RNA samples was analyzed on an RNA Pico Chip.

Biomek Automated Extraction Gave Comparable RNA Yields as Compared to Manual Extraction

To compare the RNA yield between manual extraction and Biomek automated extraction, the average RNA yield and purity was calculated from 3 batches of liver lysate prepared using the Biomek Workstation from 3 different runs. Figure 2 shows that automated extraction gave comparable RNA yields as compared to manually extracted samples.

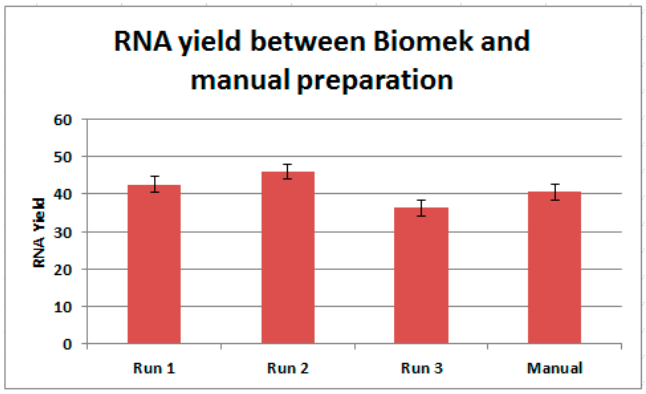

Fig. 2. Average RNA yields for Biomek automation and manual extraction methods. Y axis represents RNA yield (µg). X axis represents 3 different Biomek-extracted samples and manually extracted samples.

Biomek Automated Extraction Gave Comparable miRNA and Total RNA Recovered Efficiency as Compared to Manual Extraction

50 ng of total RNA was used to determine let-7c miRNA and B2M gene expression from either manual or Biomek automation purified RNA samples. The average cycle threshold (Ct) was calculated for each method. The average Ct value for let-7c gene expression from the Biomek-extracted samples was 23.85 ±0.05 and the manually extracted samples showed a Ct value of 24.17 ±0.024. The average Ct value for B2M gene expression from Biomek-extracted samples was 23.62 ±0.014 and the manually extracted samples showed a Ct value of 23.50 ±0.005. The result indicates that both extraction methods gave comparable miRNA and messenger RNA extraction efficiency (Figure 3). The minus RT and controls with no template showed no amplification, indicating that the amplification resulted from miRNA and mRNA alone (data not shown).

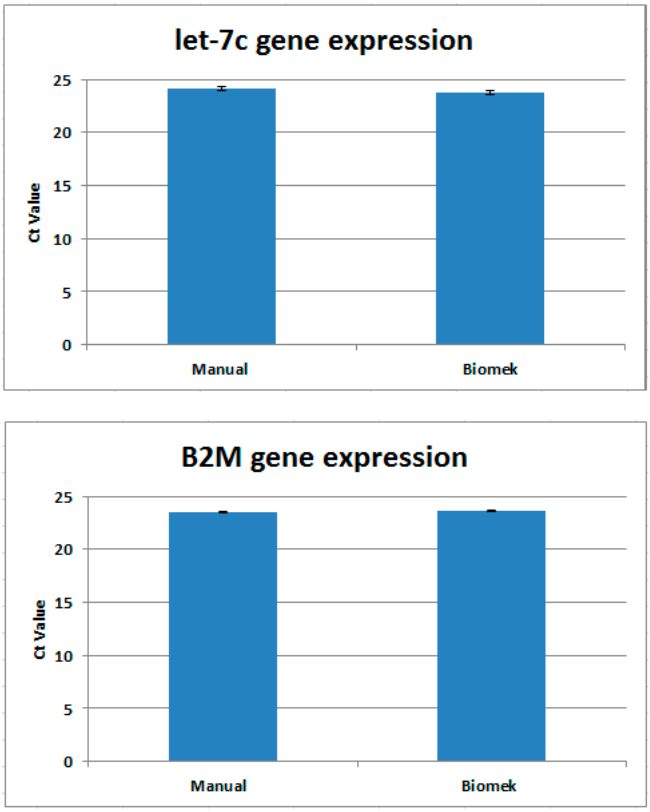

Fig. 3. Average Ct value for the let-7c miRNA and B2M gene expression in a 50 ng reaction. Results of TaqMan® gene expression assay for B2M (bottom) and microRNA assay for let-7c (top), comparing the eluates generated from the manual and automation.

Conclusions

The data from this study shows that the RNAdvance Tissue Kit provides high-purity RNA. The automated extraction and manual extraction protocols show no difference in RNA yields or miRNA and messenger RNA gene expression profiling. The Biomek RNAdvance miRNA Tissue 96 demonstrated method is an easy, robust automated nucleic acid protocol that can process from 1 to 96 samples in a 96-well plate format. It provides a streamlined workflow for downstream assays such as qPCR, microarray and NGS-RNA sequencing applications.

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Reference

1. Highly-efficient miRNA Isolation using the Agencourt FormaPure and RNAdvance Cell v2 Kits and Biomek Automated Extraction Methods.

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