Automation of Micro RNA and Total RNA Purification from tissues using the Agencourt RNAdvance Tissue Kits and Biomek NX^{P} Span 8 Laboratory Automation Workstation

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Summary

Micro RNAs are small, naturally-occurring non-coding ribonucleic acids of approximately 22 nucleotides in length that function in gene silencing and post-transcriptional regulation of gene expression. A single microRNA (miRNA) can target and regulate several (maybe even hundreds) of transcripts, and can involve multiple biological networks or pathways. As a result, interest in miRNA biomarker research has increased. This poster describes the purification of miRNA and total RNA from fresh-frozen tissue samples using the Agencourt RNAdvance paramagnetic bead based chemistry automated on the Biomek NX^{P} Laboratory Automated Workstation. The method enables automated purification of total RNA, including miRNA and other small RNAs, from 8–96 samples on a Biomek Span 8 workstation. Total RNA and miRNA can be purified from very small amounts of animal tissue as starting materials. Automating SPRI (Solid Phase Reversible Immobilization) chemistry provides an easy, high yielding and robust nucleic acid purification process that does not require centrifugation or 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 compared to samples extracted manually. The RNA yield and quality is comparable to a commonly used column method using two homogenization methods.

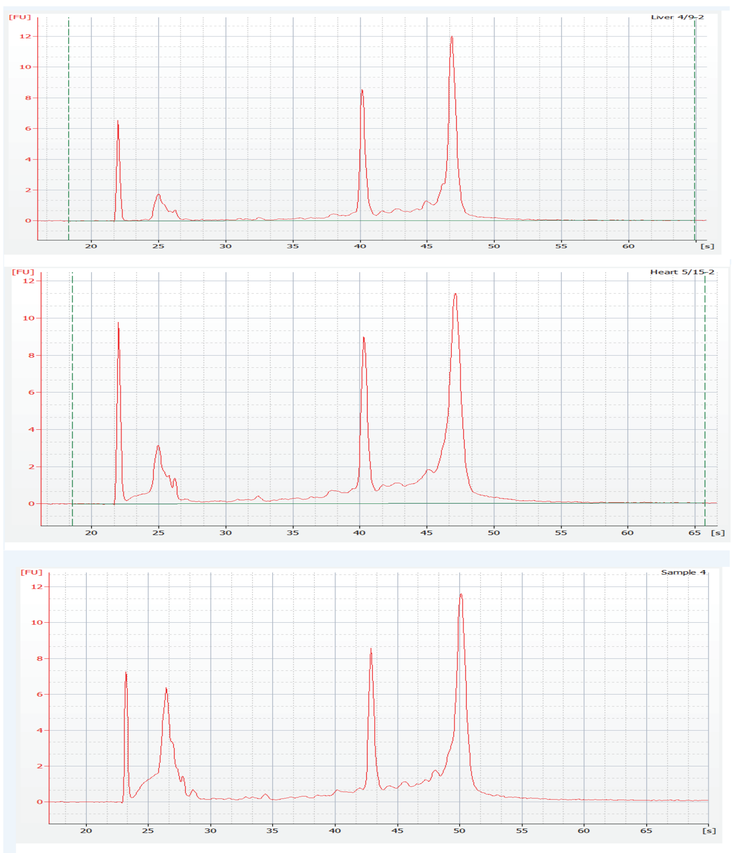
Results

Summary of RNA yield 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 Agencourt RNAdvanceTissue extraction method (Beckman, A35555) with modified bind and wash buffer steps. The average concentration from 96 samples was 1066 ng/μL with % CV of 8.6. The OD260/OD280 ratio for 96 samples ranged from 2.0-2.1. The OD260/OD230 ratio for 96 samples ranged from 1.80 -2.2.

Average conc. per 10mg (ng/μL)+/-CV%	Average yield (μg) per 10mg Tissue	Average OD 260/OD280 ratio	Average OD 260/OD230 ratio
1066+/-8.6%	42.6	2.1	2.1

Figure 1 shows examples of RNA profiling

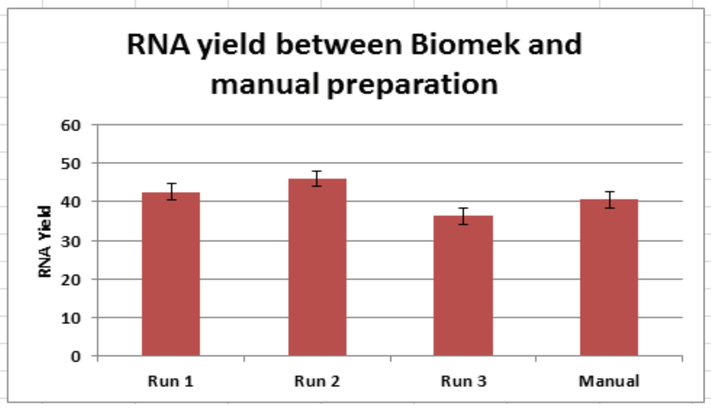


RNA Pico Chip data: 1:1000 dilution for liver (top) and heart samples (middle), and 1:300 for brain sample (bottom). The average RIN was 9.0-9.8 for liver, 9.0-9.5 for heart and 8.0-8.7 for brain tissue.

Biomek automated extraction gave comparable RNA yields 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 three batches of liver lysate prepared using the Biomek workstation from three different runs.

Figure 2 shows RNA yields between Biomek and manual preparation

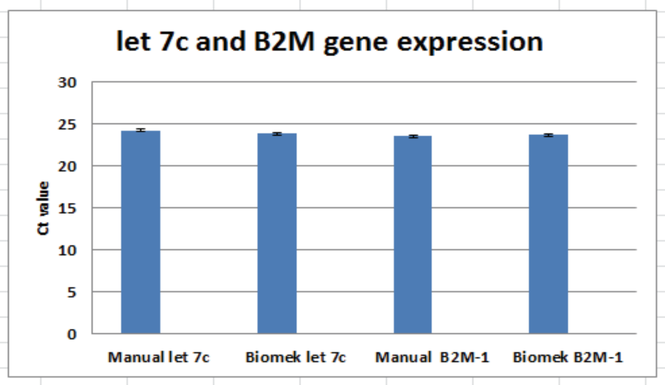


Y axis represents RNA yield (mg). X axis represents three different Biomek extracted samples and manually-extracted samples.

Gene expression data demonstrates comparable miRNA and total RNA recovery from Biomek and manual extraction methods

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).

Figure 3: Average Ct value for the let-7c miRNA and B2M gene expression in a 50 ng reaction.



Results of a Taqman gene expression assay for B2M and a microRNA assay for let 7c, comparing the eluates generated from the manual and automated protocols.

The RNAdvance Tissue purification kit gave comparable RNA yield as well as miRNA extraction efficiency compared to column purification.

A total of 16 different liver tissue replicates were used to evaluate miRNA extraction efficiency between the SPRI reagents and a common column method (miRNeasy column extraction). Two homogenization methods were used for this study (IKA Ultra Turrax tissue dispersing element and Precellys' homogenizer) in order to have an impartial comparison. RNA was extracted either using the RNAdvance Tissue Kits or miRNeasy Micro Kits. The results showed that RNAdvance Tissue Kits and miRNeasy Micro Kits gave comparable yield from both homogenization methods. The calculated average yield prepared from the RNAdvance Tissue Kit was between 24-30 μg per 5 mg liver tissue whereas the calculated average yield from the miRNeasy Micro Kit was between 19-27 μg per 5 mg liver tissue.

Table 2: The average RNA yield and quality prepared from tissue dispersing homogenizer.

Method	Yield per 5mg of liver tissue (μg)
SPRI	27.04+/-2.0
Column	21.17+/-2.0

Table 3: The average RNA yield and quality prepared from bead milling homogenizer.

Method	Yield per 5mg of liver tissue (μg)
SPRI	27.80+/-2.2
Column	23.26+/-2.5

Conclusion

The data from this study shows that the RNAdvance Tissue Kit provides high quality RNA and miRNA. The RNAdvance Tissue Kit can be used for up to 10mg per extraction, whereas the miRNeasy Micro Kit is optimized only up to 5mg tissue per extraction. The automated extraction and manual extraction protocols show no difference in RNA yields or miRNA and messenger RNA gene expression profiling. The Beckman Coulter's Agencourt RNAdvance Tissue 96 Biomek NX^{P} Span8 method is an easy, robust automated nucleic acid protocol that can process from 8 to 96 samples in a 96-well plate format. It provides a streamlined workflow for downstream assays such as qPCR, micro-array and NGS-RNA sequencing applications.

The labware and devices used in this study:

Description	QTY	Part number**
Orbital Shaker	1	379448
Span 8 Passive Wash	1	719654
Biomek 4x3 ALP Kit	1	989839
SRIPlate 96 R Ring Super Magnet Plate	`1	A32782
Reservoir Frame	1	372795
Half Reservoir	1	534681
Full Reservoir	2	372784
Quarter Reservoir	2	372790
Biomek AP96P1000 Tips	8	B01123
96-Well Riplate-2.2mL	1	Ritter, 43001-0020
Hard-shell thin-wall 96-well skirted PCR plate	1	BioRad, HSP9611

** Beckman Coulter part number