Automation of Micro RNA and Total RNA Purification from Formalin-fixed paraffin-embedded tissues (FFPE) using the Agencourt FormaPure Kit and Biomek NX^P Span 8 Laboratory Automation Workstation

Bee Na Lee, Ph.D., Staff Application Scientist and John R. Palys, Systems Engineer II
Beckman Coulter Life Sciences

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 application note describes the purification of miRNA and total RNA from FFPE tissue samples using the Agencourt Formapure paramagnetic bead based chemistry automated on the Biomek NX^P Laboratory Automation 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 10 micron FFPE slices 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 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 compared to samples extracted manually.

Materials and Methods

FFPE samples (10 micron thick slices) were de-paraffinized, lysed with proteinase K, and then extracted using the Beckman Coulter Agencourt FormaPure Kit (part number A33342). RNA was extracted according to the instructions for the FormaPure miRNA protocol AAG-666SP11.14-A (Reference 1-2) using the Agencourt FormaPure 96 Biomek NX^P Span8 method (A35556, Reference 3) with the modified buffer volume in binding and washing steps to recover miRNA. 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. 1 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 quality. Let-7c miRNA expression was determined by a Taqman microRNA assay (Life Technologies 4427975, assay ID000379). For messenger RNA gene expression, cDNA was synthesized using a B2M or ACTB gene specific reverse primer (TCTGCTCCCCACCTCTAAGT and CACCTTCACCGTTCCAGTTT respectively). PCR products were amplified using a primer probe mix cocktail. B2M (forward primer, GGACTGGTCTTTCTATCTCTTGTAC; reverse primer, ACCTCCATGATGCTGCTT AC; probe CTGCC TGTGAACCATGTGACTTTG). ACTB (forward primer ACAGAGCCTCGCCTTTG, reverse primer CCTTGCACATGCCGGAG, probe TCATCCATGGTGAGCTGGCGG). 50 ng of total RNA was used for the reverse transcription reaction using the TaqMan micro RNA Reverse Transcription kit for let-7c and 250ng for B2M and ACTB gene expression (Life Technologies,

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TYPE	QUANTITY	DESCRIPTION	PART NO.*
Devices	1 1 1	Orbital Shaker Span 8 Passive Wash Static Peltier	379448 719654 A93938
ALPS	1	Biomek NX Span-8 4x3 ALP Kit	989839
Magnet Plate	1	Agencourt SPRIPlate 96R-Ring Super Magnet Plate	A32782
Reservoirs	1 3 1 1 1	Reservoir Frame Half Reservoir Full Reservoir Quarter Reservoir Quarter Reservoir, divided by length	372795 534681 372784 372790 372788
Consumables	8 1 1	Biomek AP96 P1000 Tipboxes 96-Well Riplate-2.2 mL Hard-Shell Thin-Wall 96-Well Skirted PCR Plate	B01123 See Materials and Methods See Materials and Methods

Table 1: Biomek NX^{P} configuration (Tools and consumables). *Beckman Coulter Life Sciences.

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 set up is described in AAG-700APP11.14-B (Reference 2). For consumables and tools used for the Biomek NX^{P} Span 8 automated workstation, see table 1.

Results and Discussion

Summary of RNA yields and purity from 48 samples using the FormaPure 96 Biomek NX Span8 NX^{P} method.

48 samples (10 micron per slice) were used to evaluate RNA yield and purity. The RNA was eluted in 40 µL of nuclease-free water. The average concentration from 48 samples was 186ng/µL (Table 2). The calculated average yield for 10 micron of FFPE was 7.46 µg (Table 2). The OD260/OD280 ratio for 48 samples ranged from 1.97-2.02 with an average ratio of 2.0. The OD260/OD230 ratio for 48 samples ranged from 1.61-2.0 with an average ratio of 1.86 (Table 2). Figure 2 shows an example of the RNA profile.

Average conc. per 10 micron (ng/µL)+/-STD	Average yield (µg) per 10 micron	Average OD260/ OD280 ratio	Average OD260/ OD230 ratio
186.62+/-54	7.46	2.0	1.86

Table 2: The average yield and purity was calculated from a total of 48 different FFPE slices from the same FFPE block.

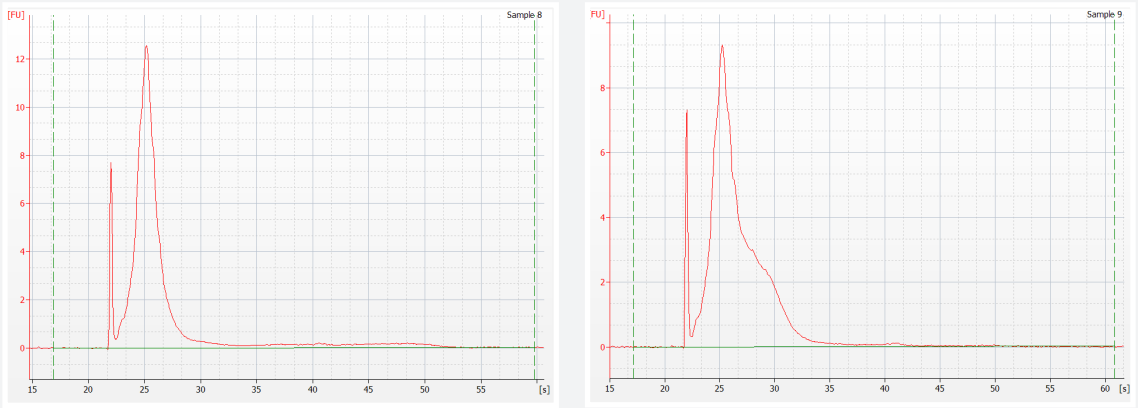

Figure 1: Example of total RNA Profiling on electropherograms. 1:30 dilution of RNA samples was analyzed on an RNA Pico Chip.

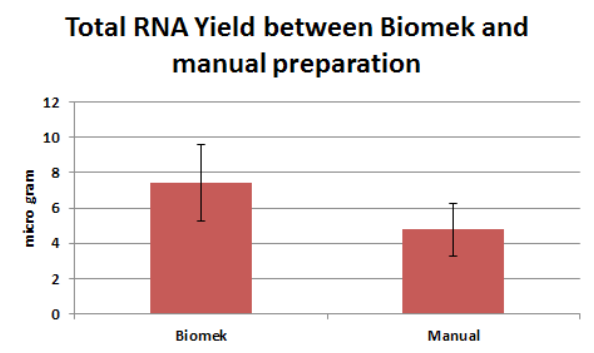

Figure 2: Average RNA yields for Biomek automated and manual extraction methods.

Biomek automated extraction gave slightly higher RNA yields compared to manual extraction.

To compare the RNA yields from manual extraction and Biomek automated extraction, the average RNA yield and purity was calculated from two batches of FFPE lysate prepared using the Biomek workstation and manual extraction. Figure 2 shows that the automated extraction gave an average of 7.4+/-2.16 µg of total RNA whereas manual extraction gave an average of 4.76+/-1.46 µg of total RNA per 10 micron individual FFPE sample. The data indicated slightly higher RNA yields for Biomek automated preparation compared to manual extraction. This is most likely due to less bead loss with extraction by the Biomek liquid handler compared to manually pipetting in multiple washing steps. Since each individual slice of FFPE samples might contain a different amount of tissue, we also observed large variation of RNA yields between samples, using either manual or Biomek extraction methods.

Gene expression data demonstrates comparable miRNA and total RNArecovery from Biomek and manual extraction methods.

50 ng of total RNA was used to determine let-7c miRNA 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 23.722+/- 0.011 and the manually-extracted samples showed a Ct value of 23.842+/-0.010. Similarly, 250 ng of total RNA was used to determine regular gene expression from either manual or Biomek automation purified RNA samples. The average Ct value for ACTB gene expression from Biomek-extracted samples was 26.06+/- 0.178 and the manually-extracted samples showed a Ct value of 26.14+/-0.019. The average Ct value for B2M gene expression from Biomek-extracted samples was 26.42+/- 0.117 and the manually-extracted samples showed a Ct value of 26.60+/-0.062. 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).

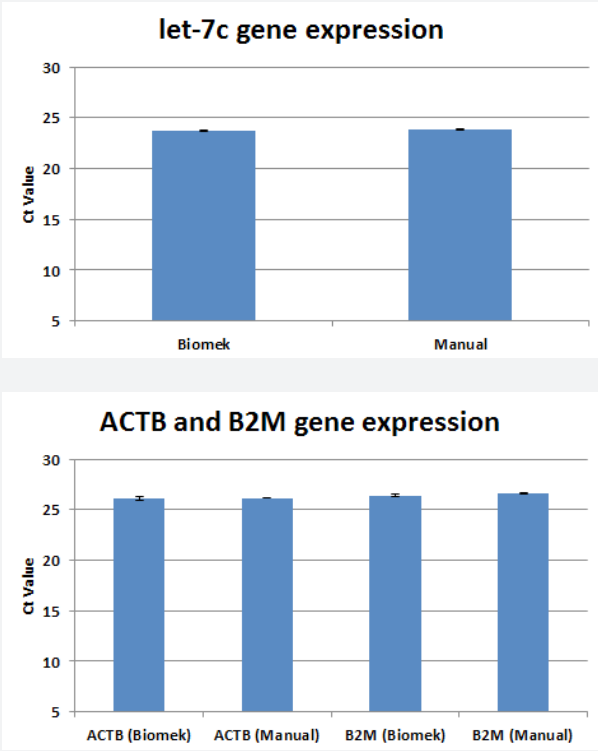

Figure 3: Average Ct value for the let-7c miRNA and regular gene expression. Results of theTaqman gene expression assay for microRNA assay for let 7c (top) and ACTB and B2M (bottom) comparing the eluates generated from the manual and automation extraction methods.

Conclusions

The data from this study shows that the FormaPure Kit provides high quality RNA. The automated extraction and manual extraction protocols show similar miRNA and messenger RNA gene expression profiling. The FormaPure 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.

Reference

1. FormaPure® Kit Supplemental Protocol for microRNA and total RNA Isolation from Formalin-Fixed, Paraffin-Embedded Tissue (AAG-666SP11.14-A).
2. Highly efficient microRNA and total RNA purification from Formalin-fixed paraffin-embedded tissues (FFPE) using Agencourt FormaPure Kit (AAG-700APP11.14-B).
3. Automated TruSeq RNA sample preparation from FFPE tissue specimens utilizing the Biomek FX^{P} liquid handler. IB-17929A

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AAG-747APP01.15-A