Micro RNA and Total RNA Purification from Blood Stabilized in PAXgene Blood RNA Tubes using the Agencourt RNAdvance Blood Kit

Introduction

The RNA content and gene expression profiles of blood samples provide useful information to study and understand both systemic and tissue-specific pathological changes. Here we describe an RNAdvance Blood kit supplemental protocol, that can be used to extract miRNA and total RNA from blood stabilized in PAXgene Blood RNA Tubes. This method uses Beckman Coulter's SPRI (Solid Phase Reverse Immobilization) magnetic bead-based chemistry—which provides an easy, rapid, high yielding, robust and automation-friendly nucleic acid purification procedure that does not require vortexing, centrifugation or filtration steps. This data shows that the RNAdvance Blood method produces high-quality miRNA and RNA in one eluate from PAXgene stabilized blood samples.

Materials and Methods

Blood was collected in PAXgene Blood RNA Tubes (PreAnalytiX) from consenting healthy adults, stored for 20–24 hours at room temperature and frozen at -20°C. The tubes were thawed for 2 hours at room temperature before processing. 400 µL of PAXgene blood was digested with 300 µL lysis buffer containing 20 µL of proteinase K at 37°C for 15 minutes. RNA was extracted following the instructions for the RNAdvance Blood miRNA supplemental protocol (Beckman Coulter document #AAG-567SP10.14-A). The 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 1:10 diluted RNA sample was analyzed by an Agilent RNA 6000 Pico chip (Agilent Technologies, 5067-1513) using the 2100 Bioanalyzer (Agilent Technologies) to determine RNA integrity. miR16, let7c and RNU44 miRNA gene expression was determined by TaqMan® microRNA assay (Life Technologies 4427975, assay ID002171, assay ID000379 and assay ID001094 respectively). For messenger RNA gene expression, cDNA was synthesized using a B2M, ACTB or HPRT1 gene specific reverse primer (TCTGCT-CCCCACCTCTAAGT, CACCTTCACCGTTCCAGTTT and AACAATCCGCCC AAAGGGAA respectively). PCR products were amplified using a primer probe mix cocktail. B2M (forward primer, GGACTGGTCTTTCTA-TCTCTTGTAC; reverse primer, ACCTCCATGATGC-TGCTT AC; probe CTGCC TGTGAACCATGTGACTTTG). ACTB (forward primer ACAGAGCCTCGCCTTTG, reverse primer CCTTGCACATGCCGGAG, probe TCATCCATGGTGAGCTGGCGG). HPRT1 (forward primer AGA TGGTCAAGGTCGCAAG, reverse primer GTATTCATTATAGTCAAGGGCATATCC, probe TGGTGAAAAGGACCC CACGAAGT). 50 ng of total RNA was used for the reverse transcription reaction using the TaqMan® microRNA Reverse Transcription kit (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).

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Results and Discussion

Summary of RNA Yields from PAXgene Blood Using the RNAdvance Blood Kits

Four PAXgene Blood samples from 4 different donors were used to evaluate RNA yield, purity and quality. A total of 12 samples were extracted for total RNA and miRNA. The RNA was eluted in 40 µL of nuclease free water. The results show that the average RNA concentration was at 29.18+/-3.56 ng/µL (range between 21.3 µg/µL to 33.6 µg/µL) with an average yield at 1.2 µg (range between 852 ng to 1344 ng). The average OD260/OD280 ratio for all 12 samples was at 2.1 (range between 2.05–2.2), and the average OD260/OD230 ratio was at 1.8 (range between 1.42–1.98). The RIN (RNA Integrity Number) scores for 12 samples were from 7.0–8.5 (Table 1).

Table 1. The Average Yield and Purity. Calculated from a total of 12 samples from 4 different donors.

Average Concentration (ng/µL)	Average Yield (µg)	Average OD260/ OD280	Average OD260/ OD230	RIN Scores
29.18+/-3.56	1.2+/-0.16	2.1+/-0.05	1.8+/-0.14	7.0-8.5

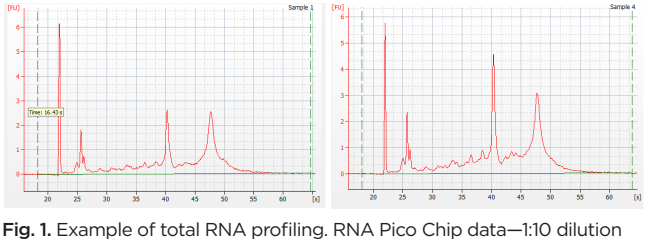

Fig. 1. Example of total RNA profiling. RNA Pico Chip data—1:10 dilution PAXgene Blood RNA samples.

Micro RNA and Messenger RNA Gene Expression Indicating miRNA and Total RNA Were Successfully Extracted from PAXgene Blood Samples

50 ng of total RNA was used to determine miRNA and messenger RNA gene expression. For miRNA gene expression, let-7c, miR16 and RNU44 were used for evaluation. The average cycle threshold (Ct) was calculated from triplicates in each donor. The average Ct value for let-7c gene expression was 22.13+/-0.024, miR16 gene expression was 16.77+/-0.004 and RNU44 gene expression was 22.47+/-0.004 (Figure 2). The minus RT and controls with no template showed no amplification, indicating that the amplification resulted from miRNA alone (data not shown). For messenger RNA gene expression, B2M, ACTB and HPRT1 genes were used for evaluation, with the results shown in Figure 3. The average Ct value for B2M gene expression was 22.98+/-0.015, ACTB gene expression was 24.69+/-0.042 and HPRT1 gene expression was 31.85+/-0.045. The minus RT and controls with no template showed no amplification, indicating that the amplification resulted from messenger RNA alone (data not shown).

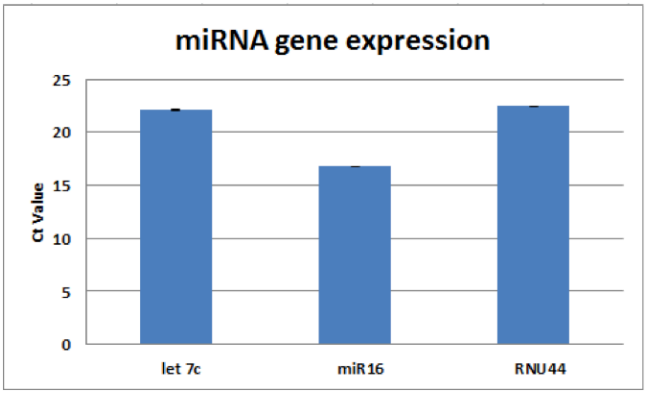

Fig. 2. Average Ct value for the let-7c, miR16 and RNU44 gene expression. The average Ct value was calculated from two different samples from the same donor.

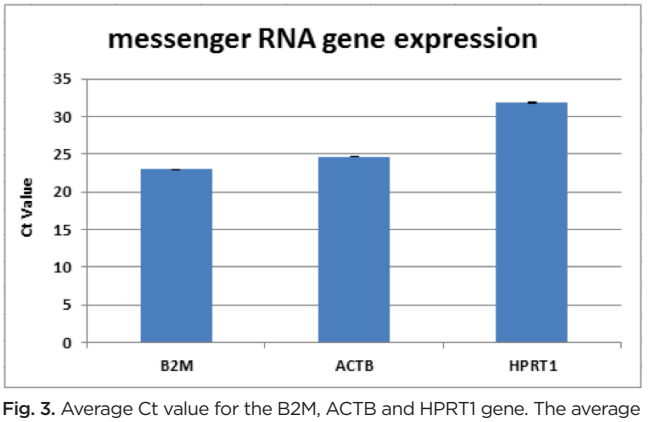

Fig. 3. Average Ct value for the B2M, ACTB and HPRT1 gene. The average Ct value was calculated from two different samples from the same donor.

Conclusions

The data from this study demonstrates that the RNAdvance Blood Kit provides high-quality RNA and miRNA from PAXgene stabilized blood samples. The magnetic bead-based extraction protocol provides automation-friendly, scalable throughput and does not require vortexing, centrifugation, or filtration steps.

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