Micro RNA and Total RNA Purification from Tissues using the AgencourtRNAdvance Tissue Kit

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Introduction

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. Here we describe the purification of total RNA, including miRNA and other small RNA molecules from fresh frozen tissue samples using the Agencourt SPRI (Solid Phase Reverse Immobilization) magnetic bead based chemistry. Extracting RNA from tissues often involves phenol and chloroform as well as multiple centrifugation steps. The SPRI method is an easy, rapid, high yielding, robust and automation-friendly nucleic acid purification procedure that does not require vortexing, centrifugation or filtration steps. The RNAdvance Tissue protocol has additional steps for lysing and purifying the sample. This technical note demonstrates that the SPRI extraction method produces high quality miRNA and RNA using two homogenization methods. The RNA yield and quality is comparable to a commonly used column method.

Materials and Methods

Rat liver, brain and heart tissue were homogenized using either a Precellys 24 (Bertin) or Ultra Turrax (IKA) homogenizer. For the Precellys homogenization, 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 antifoaming Dx Reagent (Qiagen, 19088). The homogenization conditions are as follows: 6500 rpm for 20 sec at the 1st cycle and 6000 rpm for 20 sec at the 2nd cycle. For the Ultra Turrax tissue dispersing device, 100 mg of tissue in 4 mL of lysis buffer was homogenized at the highest speed for 60 seconds or until the tissue was homogenized completely. Lysate was adjusted to 10 mg per 400 µL lysis buffer and then digested with proteinase K at 37˚C for 25 minutes. RNA was extracted according to the instructions for the RNAdvance Tissue miRNA protocol (www.beckman.com, AAG-230APP07.14-A) using the tube format method. For the miRNeasy Micro kit (Qiagen, 217084) samples, 50-100 mg of rat liver tissue was homogenized using either Precellys 24 (Bertin Technologies) or the Ultra Turrax dispersing element as described above. Lysate was adjusted to 5mg per 700 µL Qiazol buffer and then RNA was extracted according to the miRNeasy Micro Kit protocol (Qiagen, 217084).

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 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. Let-7c miRNA gene expression was determined by Taqman microRNA assay (Life Technologies 4427975, assay ID000379). 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 three different tissue types using the RNAdvance Tissue Kits.

An average of 10 mg of three tissue types was used to evaluate RNA yield. Liver (soft), heart (fibrous) and brain (lipid rich) tissue was prepared for RNA extraction. Eight samples from each type of tissue were extracted for total RNA and miRNA. The RNA was eluted in 40 µL of nuclease free water. The results show that liver and heart tissue gave at least three times higher RNA yields as compared to more difficult brain tissue (lipid rich) (Table 1). All samples show high RNA quality as indicated by RIN (RNA Integrity Number) scores (Table 2). The lower RIN scores observed in brain samples that could have resulted from the higher amounts of smaller RNA in the samples as indicated by the relatively larger area of small RNA present in the electropherograms. The software has no way to detect that the fluorescence in this area is due to enrichment for small RNA and calculates it as degraded RNA (Figure 1). Figure 2 shows the examples of RNA profiling from the liver, heart and brain. The OD260/OD280 ratio for all 24 samples was above 1.9, and the OD260/OD230 ratio was above 1.2 (data not shown).

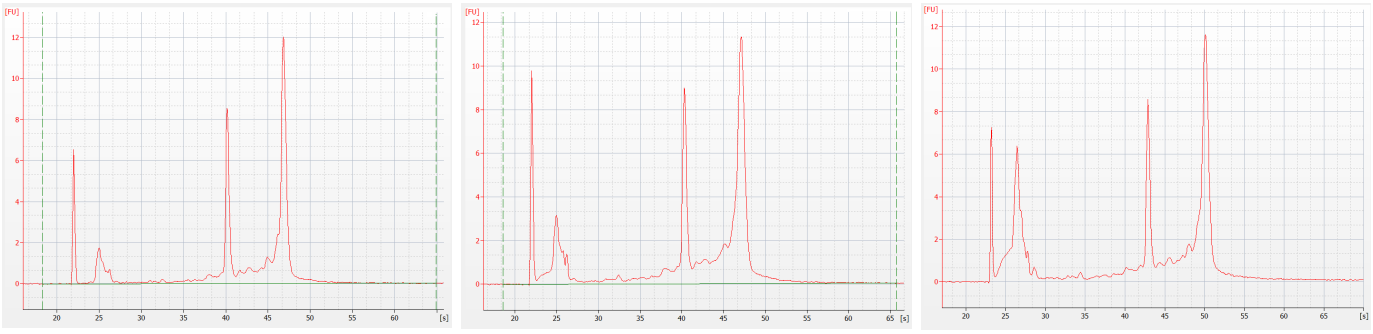
TISSUE	LIVER	HEART	BRAIN
Average Yield (µg) per 10 mg	53.70 +/- 4.0	46.6 +/- 2.8	14.72 +/- 4.5

Table 1: The average yield was calculated from a total of 8 samples for each tissue type.

TISSUE	LIVER	HEART	BRAIN
RIN	9.0-9.8	9.0-9.5	8.0-8.70

Table 2: The average RIN was calculated from a total of 8 samples for each tissue type.

Figure 1. Example of total RNA Profiling:

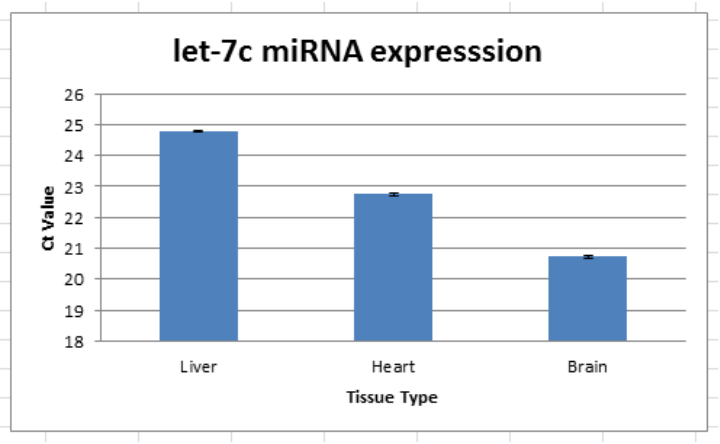


RNA Pico Chip data: 1:1000 dilution for liver (left) and heart samples (middle) and 1:300 for brain sample (right).

Micro RNA let-7c gene expression in liver, heart and brain tissues.

50 ng of total RNA was used for let-7c gene expression to determine miRNA gene expression. The results show that brain tissue expressed let-7c miRNA more abundantly than either liver or heart tissue (Figure 2). The average cycle threshold (Ct) was calculated from three samples in each tissue type. The average Ct value for let-7c gene expression in the brain was 20.72+/-0.038, heart was 22.75+/-0.038 and liver was 24.78+/-0.022. The minus RT and controls with no template showed negative amplification, indicating that the amplification resulted from miRNA alone.

Figure 2. Average Ct value for the let-7c gene expression in a 50 ng reaction



The average Ct value was calculated from three samples extracted from different experiments.

The RNAdvance Tissue purification kit gave comparable RNA yield as well as miRNA extraction efficiency as 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 3 and 4). The average cycle threshold (Ct) value for the let -7c miRNA expression in RNA samples prepared from both methods was at 25 cycles, indicating that both extraction methods gave similar miRNA extraction efficiency (Figure 3).

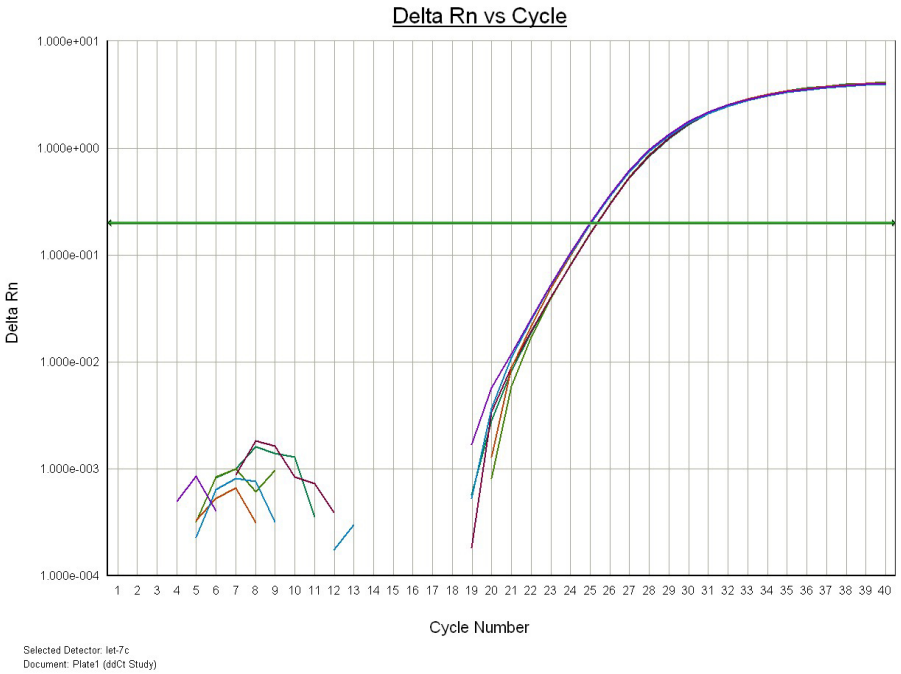
METHOD	YIELD PER 5 mg LIVER TISSUE (µg)	AVERAGE YIELD PER 5 mg LIVER TISSUE (µg)
RNAdvance Tissue	24-30	27.04 +/-2.0
miRNeasy	19-23	21.17+/-2.0

Table 3: The average RNA yield and quality prepared from tissue dispersing homogenizer

METHOD	YIELD PER 5 mg LIVER TISSUE (µg)	AVERAGE YIELD PER 5 mg LIVER TISSUE (µg)
RNAdvance Tissue	25-30	27.80+/-2.2
miRNeasy	21-27	23.26+/-2.5

Table 4: The average RNA yield and quality prepared from Precellys homogenizer.

Figure 3. Overlay of let-7c gene expression from the RNAdvance Tissue Kits and miRNeasy Micro Kits.



Taqman qPCR amplication plots show that both extraction methods gave a Ct at 25 cycles.

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 10 mg per extraction, whereas the miRNeasy Micro Kit is optimized only up to 5 mg tissue per extraction (see miRNeasy protocol). The magnetic bead based extraction protocol does not require centrifugation, filtration or precipitation steps and the use of phenol chloroform. It provides scalable throughput and it is automation-friendly.