Highly Efficient MicroRNA and Total RNA Purification from Formalin-Fixed Paraffin-Embedded Tissues (FFPE)

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Introduction

Extracting miRNA and total RNA from highly degraded archival formalin-fixed, paraffin-embedded tissue (FFPE) can be challenging due to combinations of fixation conditions, age, temperature, and storage. In the past few years, interest in the identification, detection and use of small RNA molecules from FFPE samples has rapidly expanded. The small RNA molecules refer to small interfering RNAs (siRNA), non-coding small RNA and regulatory microRNAs (miRNAs). These small RNA molecules, which range between 15-40 nucleotides in length, are not efficiently extracted by traditional precipitation or solid phase methodologies. This technical note describes how the Agencourt SPRI (Solid Phase Reverse Immobilization) magnetic bead based method is superior to the column purification method for purifying miRNA from formalin-fixed paraffin-embedded tissues (FFPE). The SPRI method is an easy, rapid, high yielding, robust and automation-friendly nucleic acid purification procedure that does not require centrifugation or filtration steps. This method uses carboxyl-coated magnetic particles that reversibly bind nucleic acids in the presence of binding buffers and crowding reagents. Typically, there are three basic steps in the extraction/purification procedure. In the first step, nucleic acids are immobilized onto the SPRI beads, leaving contaminants in solution. In the second step, a magnetic field is used to attract the micro-particles with bound nucleic acids out of the solution. Contaminants are then aspirated and the micro-particles with bound nucleic acids are thoroughly washed with molecular biology-grade ethanol. In the third step, purified nucleic acids are easily eluted from the micro-particles under low salt aqueous conditions, which provide maximum flexibility for downstream applications. The results show that the SPRI extraction method provides 5 times higher miRNA yield than the column purification method.

Materials and Methods

FFPE samples (four 10 micron thick slices) were deparaffinized, lysed with proteinase K digestion, and then extracted using either the Agencourt FormaPure Kit (Beckman Coulter, A33342) or miRNeasy FFPE Kit (Qiagen, 217504). The lysate was prepared and the purification steps were performed manually according to the vendor instructions for the miRNeasy kit and the FormaPure miRNA protocol described in the application note (www.beckman.com, AAG-666SP11.14-A) using the tube format (Beckman Coulter Life Sciences, A29182). In order to have a fair comparison of the RNA yields between these two methods, all sixteen samples were eluted in 40 µL of nuclease free water in the final elution step. The concentration and purity of the RNA was measured by using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Purity was determined by the ratio of the OD260/OD280 and OD260/OD230. 1 µL of total RNA was analyzed by using the Agilent RNA 6000 Pico chip (Agilent Technologies, 5067-1513) and the 2100 Bioanalyzer (Agilent Technologies). microRNA gene expression was determined by Taqman microRNA assay (Life Technologies 4427975, assay ID000379). 50 ng and 100 ng of total RNA was used for the reverse transcription reaction using the TaqMan micro RNA 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

Comparison the RNA yield: FormaPure purification kit gave a higher RNA yield than column purification kits.

To compare the miRNA and RNA yield between the FormaPure and Qiagen miRNeasy purification methods for the FFPE samples, eight of the FFPE samples were processed using the FormaPure kit and an additional eight were processed using the Qiagen miRNeasy FFPE kit. The result from Table 1 shows that the SPRI bead purification method produced average yields 5 times higher than the column purification method. The average yield prepared from the FormaPure kit was 10 µg and the average yield from the miRNeasy FFPE kit was 2 µg. The average yield per 10 micron of FFPE sample for the FormaPure kit was 2.5 µg whereas miRNeasy FFPE kit produced only 0.5 µg per 10 micron of FFPE sample. The average RNA concentration for the FormaPure kit was 257 ng/µL and the average concentration of RNA for the miRNeasy FFPE kit was at 48 ng/µL (Table 1).

Table 1: Summary of the average RNA yield:

Method	Average Conc. (ng/µL)	Average Yield (µg)	Elution Volume (µL)
FormaPure	257.0	10.0	40
miRNeasy	48.0	1.92	40

A total of 8 liver samples were evaluated per method.

Comparison of RNA purity: Similar RNA purity and quality were produced by both FormaPure and the column purification kits.

Figure 1 shows the typical fragmented FFPE RNA profile extracted using both methods. For the FormaPure extracted samples, the total RNA was diluted to 1:50 and miRNeasy samples were diluted 1:10 in order to get equivalent signals. All samples have similar typical FFPE RNA profiling with the low RIN between 2.3-2.4. The RNA purity was evaluated from a total of 24 samples (12 samples per method), showing that the RNA purity was consistent between these two methods. The OD260/OD280 ratio for both methods was between 1.8-2.0, with the average ratio above 1.8. The OD260/OD230 ratio for both methods was between 1.0-2.10 with the average ratio above 1.6 (Tables 2-1 and 2-2).

Table 2-1: Summary of RNA purity:

Method	Average OD260/OD280	Range OD260/OD280
FormaPure	1.89	1.83-2.04
miRNeasy	1.87	1.76-1.96

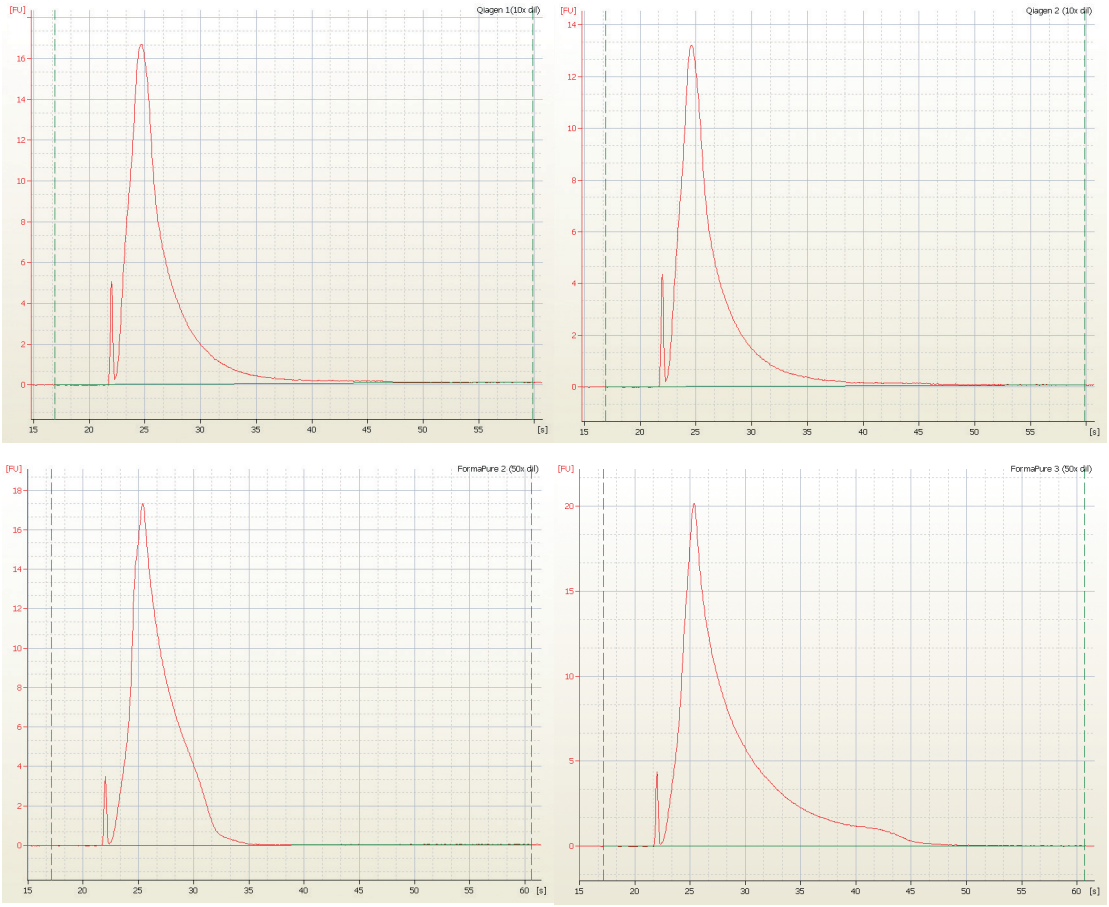
A total of 12 samples were evaluated per method. Sample type includes liver, colon and breast.

Table 2-2: Summary of RNA purity:

Method	Average OD260/OD230	Range OD260/OD230
FormaPure	1.65	1.02-2.10
miRNeasy	1.72	1.04-2.03

A total of 12 samples were evaluated per method. Sample type includes liver, colon and breast.

Figure 1. Typical fragmented FFPE RNA profiling:



RNA Pico Chip data: 1:10 dilution of the miRNeasy RNA samples (upper panel) and 1: 50 dilution of the FormaPure RNA samples (lower panel).

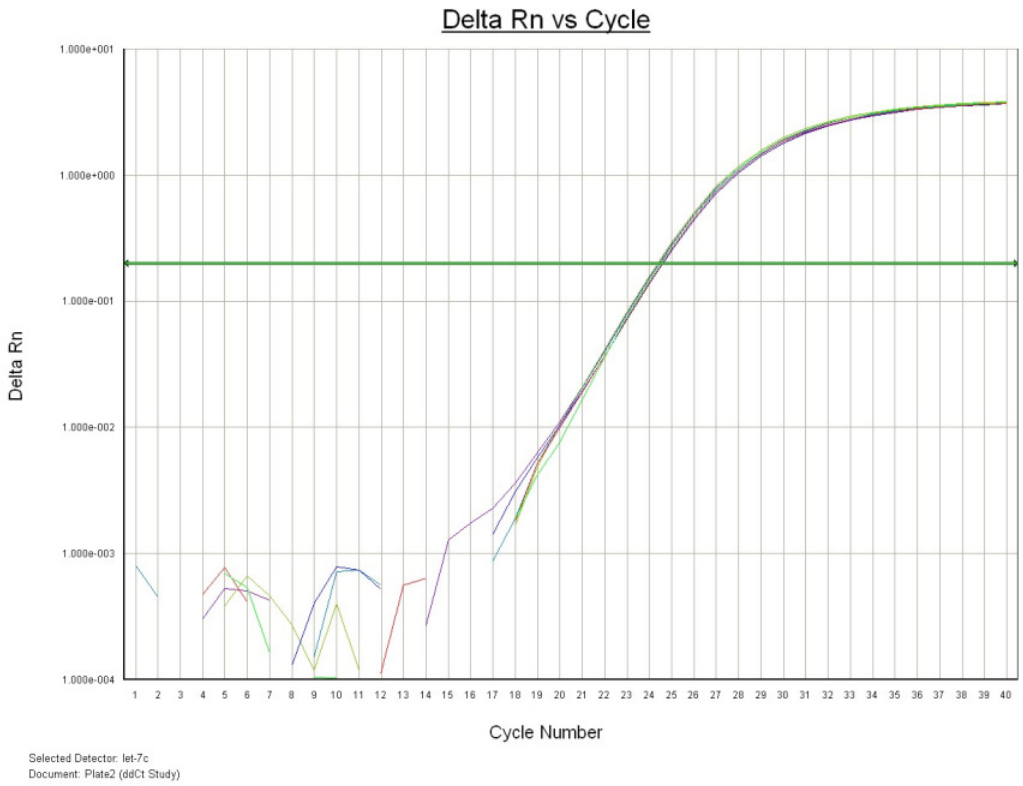
Comparison of miRNA yield: FormaPure purification showed higher miRNA extraction efficiency.

To determine the miRNA extraction efficiency, two different concentrations of total RNA from four liver samples extracted from both FormaPure and miRNeasy kits were used for let-7c gene expression. The results show the average threshold cycle value (Ct) for all samples was 23.5 Ct for 100 ng per reaction and 24.5 Ct for 50ng per reaction (Table 3 and Figure 2). The minus RT and controls with no template showed negative amplification, indicating that the amplification resulted from miRNA alone. This data confirmed that 5 times more miRNA was extracted using the FormaPure kit compared to the miRNeasy, because about 1/5 as much RNA was needed for cDNA synthesis in the reverse transcription reactions.

Table 3: Average Ct value for the let-7c gene expression

Kit Used	Amount of RNA Used	Average Ct value+/- Std Dev
FormaPure	100 ng	23.81+/-0.004
FormaPure	50 ng	24.57+/-0.029
miRNeasy	100 ng	23.45+/-0.035
miRNeasy	50 ng	24.62+/-0.035

Figure 2. Overlay of let-7c gene expression from liver FFPE samples extracted from FormaPure and miRNeasy kits.



The Taqman qPCR amplication plots showed that 1/5 as much RNA was needed from the miRNeasy extracted samples in order to achieve the same cycle threshold as those extracted using the Formapure kit..

Conclusions

The data from this study showed that miRNA and total RNA extracted from both the FormaPure kit and the miRNeasy kit had comparable purity and quality. However, the FormaPure kit gave RNA yields 5 times higher than the miRNeasy kit. Therefore, the FormaPure kit provides a more robust miRNA and total RNA extraction method for critical FFPE samples.

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