RNA Isolation from FFPE Tissues – Comparing the Performances of Three Commercial Kits

*Hong Zhao, Nicholas R. Bertos, and Morag Park**
Cancer Centre, McGill University, Montreal, Quebec, Canada
**Corresponding author: morag.park@mcgill.ca*


Morag Park

In order to compare the performance of the High Pure FFPE RNA Micro Kit with that of two FFPE RNA isolation kits from other manufacturers, total RNA was isolated from sections of mouse mammary tumors with each of the three kits. Yield and integrity of the isolated RNA as well as genomic DNA (gDNA) contamination were assessed. Real-time RT-PCR of the housekeeping gene ACTB was performed to evaluate performance of the kits. In terms of yield and the $A_{260/280}$ and $A_{260/230}$ ratios, kits A and B seemed to perfom better than the High Pure FFPE RNA Micro Kit. In terms of real-time RT-PCR sensitivity, however, the High Pure FFPE RNA Micro Kit showed superior results.

Introduction

Archived formalin-fixed, paraffin-embedded (FFPE) tissue samples are extremely valuable material for conducting retrospective studies. However, isolation of RNA from FFPE samples is a technical challenge due to the cross-linking and modification introduced during the tissue fixation and embedding procedures. High RNA quality is vital for downstream applications, and the quality of the RNA obtained is dependent largely on the RNA isolation procedure used. In order to assess the ability of three commercially available kits to obtain high-quality RNA from FFPE samples for downstream real-time RT-PCR analysis, we carried out a set of preliminary comparative trials.

Materials and Methods

We assessed the High Pure FFPE RNA Micro Kit and two other commercially available FFPE RNA isolation kits (A and B). Each kit is based on similar principles: FFPE samples are initially deparaffinized, followed by cell-lysate treatment with proteinase K at high temperature (55–70°C). Double-stranded DNA is then degraded using an in-column DNase I digestion method, or removed with a selective column technology.

A formalin-fixed paraffin-embedded mouse mammary tumor block was sectioned at 5 µm thickness using a microtome. Total RNA was isolated from two sections (each having a tissue surface area of 48 mm^2) with each kit. RNA concentration was measured with an ND-1000 spectrophotometer (NanoDrop) and RNA integrity (RIN, RNA Integrity Number) was assessed using a commercially available kit run on an Agilent Bioanalyzer 2100.

To assess gDNA contamination in the isolated RNA samples, a dilution series of mouse genomic DNA (2 ng, 0.1 ng, 0.01 ng, 0.001 ng) was prepared as standards. 100 ng of the RNA samples isolated using each of the three kits was used as template, omitting the reverse transcription step. Real-time PCR with GAPDH primers (designed within the same exon) was performed using a commercially available kit. gDNA contamination was determined from the standard curve.

To evaluate the downstream performance of the total RNA isolated with the three different kits, a real-time RT-PCR for the housekeeping gene ACTB was performed using a commercially available kit on the LightCycler® Carousel-Based System. The amplicon length of the ACTB product is 77 bp.

Results and Discussion

The yield of total RNA isolated with kits A and B was higher than that obtained with the High Pure FFPE RNA Micro Kit, and the $A_{260/280}$ and $A_{260/230}$ ratios also appeared to be superior (Table 1). However, the High Pure FFPE RNA Micro Kit demonstrated the best performance compared with the other two kits in a downstream quantitative RT-PCR application. The crossing point (Cp) of ACTB RT-PCR with High Pure FFPE RNA Micro Kit-derived RNA appeared more than one cycle earlier than with the other kits (Table 2). Thus, the question arises as to whether measuring $A_{260/A280}$ and $A_{260/A230}$ ratios is enough to identify high-quality RNA. It has been demonstrated that the $A_{260/A280}$ ratio is very sensitive to changes in the pH and ionic strength of the RNA-contain-

Table 1: RNA isolation results.

Type of FFPE RNA kit	Concentration (ng/µl)	$A_{260/280}$	$A_{260/230}$	Yield (ng)	RIN
High Pure FFPE RNA Micro Kit (1)	16.28	1.77	0.17	260.48	1.8
High Pure FFPE RNA Micro Kit (2)	29.30	1.84	0.58	587.80	2.0
Kit A (1)	91.24	2.04	0.76	1368.60	2.2
Kit A (2)	81.73	2.05	0.43	1225.95	2.0
Kit B	69.15	2.04	1.95	691.50	2.0

Table 2: Real-time RT-PCR Results. Results are those of a representative sample from duplicate experiments.

FFPE RNA isolationKit	Cp* ACTB
High Pure FFPE RNA Micro Kit	24.05
Kit A	25.48
Kit B	25.20

*Crossing point (Cp) value is inversely related to transcript abundance

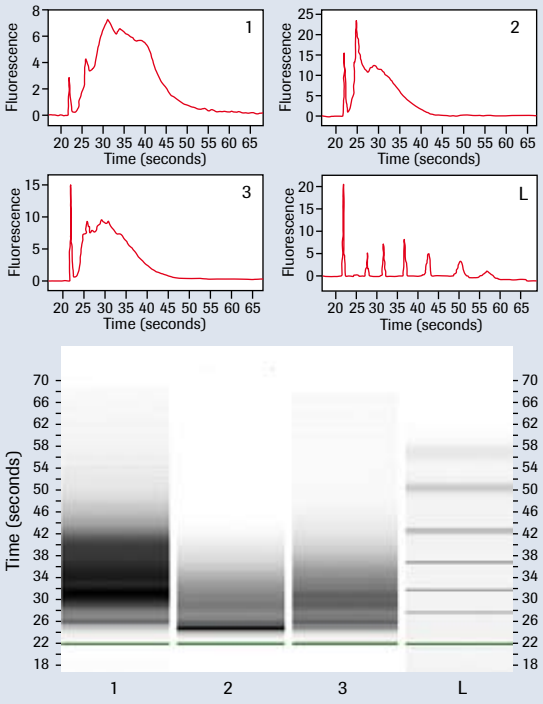

Figure 1: Bioanalyzer profiles. 1 = High Pure FFPE RNA Micro Kit; 2 = Kit B; 3 = Kit A; L = Ladder.

ing solution. The A_{260} reading in standard solutions (pure water, TE, or Tris) is relatively consistent, independent of pH and ionic strength, while low pH and/or low ionic strength increase the absorbance of some contaminants (*i.e.*, protein) at 280 nm, thus lowering the $A_{260/280}$ ratio [1, 2]. This may be more evident at lower RNA concentrations. Since the High Pure FFPE RNA Micro Kit uses pure water as RNA solvent, and the RNA concentration is lower (16.28 ng or 29.3 ng/µl), it is expected that the $A_{260/A280}$ ratio observed with the High Pure FFPE RNA Micro Kit-derived RNA is lower than that seen for RNA derived using kits A and B.

The $A_{260/230}$ ratio, which indicates the amount of organic contamination in isolated RNA samples, is much more variable than the $A_{260/A280}$ ratio, and thus does not have a significant correlation with downstream applications. Interestingly, the kit B-derived RNA showed little gDNA contamination in contrast to the other two RNAs (Table 3). While kit B uses a column-based selective removal method, the High Pure FFPE RNA Micro Kit and kit A both use an in-column DNase

Table 3: gDNA contamination. RNA was isolated using three different kits.

FFPE RNA isolation kit	gDNA in 100 ng RNA*
High Pure FFPE RNA Micro Kit	0.300 ng
Kit A	0.380 ng
Kit B	0.003 ng

*Results are those of a representative sample from duplicate experiments

I treatment procedure. We also performed real-time RT-PCR with GAPDH primers designed inside the same exon. The results showed that the gene expression pattern of GAPDH (data not shown) is exactly the same as ACTB (Table 2), for which primers were designed within different exons to prevent PCR amplification from gDNA. Thus the gDNA contamination level detected in RNA isolated with the High Pure FFPE RNA Micro Kit or kit A does not appear to significantly affect RT-PCR assays in these preliminary experiments.

The RIN is comparable among the three kits (Table 1). The fragment size of High Pure FFPE RNA Micro Kit-derived total RNA, according to the Bioanalyzer profile, was larger and wider (ranging from 200 to 1,000 nucleotides) than the other two kits (which ranged from less than 200 to 500 nucleotides) (Figure 1). The RIN is well accepted as a measure for quality control in downstream applications. It is recommended that for PCR applications, total RNA RIN should be higher than 5, and for microarray applications higher than 7 or 8 [3]. However, it is not very meaningful in respect to the typical highly degraded FFPE-derived RNA. An RIN value of 1.4 has been used as a cut-off limit for biologically useful RNA in one study where RNA quality (RIN>1.4) from 10-year-old FFPE samples was similar to that seen in months-old samples, but quantity and success rate were generally higher for the months-old group [4]. In our assay, the short length of the ACTB amplicon (77 bp) would also help to avoid the effect of poor RNA integrity on PCR performance.

Conclusions

RNA isolated from FFPE tissue samples using the High Pure FFPE RNA Micro Kit showed a higher real-time RT-PCR sensitivity than RNA isolated using two other commercially available kits. ■

References

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