Application Forum

Purification of Quality RNA from FFPE Tissue Using the ReliaPrep™ FFPE Total RNA Miniprep System

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Introduction

Purification of quality, amplifiable RNA from formalin-fixed paraffin-embedded (FFPE) tissue can be a difficult task. The process of fixation can be extremely harsh and may cause fragmentation of nucleic acids present within the sample. RNA is particularly susceptible to fragmentation, given its inherent fragility. Fixation also introduces cross-linkages which can hamper the processivity of many downstream applications. The storage conditions of FFPE samples can also play a role in the quality of RNA present within the sample. Archival FFPE tissue blocks and slides may be decades old and have been subjected to conditions that have compromised sample integrity.

FFPE samples are of great interest to researchers across many fields and application areas. In order to apply RNA from FFPE tissue to sensitive downstream applications, many researchers purify the RNA using home brew techniques or commercially available kits that involve harsh organic solvents for sample deparaffinization and long, overnight digestions to remove any proteins present in the sample. These techniques can cause further RNA damage and result in purified product that is not suitable for downstream applications such as RT-qPCR, microarrays, or sequencing. Gentle purification and accurate sample analysis are critical when dealing with RNA from FFPE tissues. While there may seem to be adequate amounts of RNA, as determined by spectrophotometry, the RNA purified by these methods may be fragmented to an extent that it is unusable in downstream assays.

In this study, we evaluated the functional yield (quantity, degree of fragmentation, and ability to be amplified) of RNA purified from FFPE tissue using two different commercially available miniprep kits: the Promega ReliaPrep™ FFPE

Total RNA Miniprep System and the Qiagen RNeasy FFPE Kit. Both methods utilize spin column technology and do not require overnight digestions. The RNeasy kit requires a separate purchase of a non-organic deparaffinization reagent or traditional xylene to perform the deparaffinization step. The ReliaPrep™ FFPE Total RNA Miniprep System includes the non-organic deparaffinization reagent in the kit.

Materials and methods

In order to test the functional yield and sample quality, total RNA from sequential 10µm mouse liver FFPE sections were purified per the manufacturer's instructions (Promega technical manual TM353, Qiagen RNeasy FFPE Handbook) and analyzed using spectrophotometry (NanoDrop) and via the Agilent Bioanalyzer per the manufacturer's instructions.

To further examine fragmentation of RNA, human liver sections, obtained commercially from BioChain, were processed using the same kits as previously described. All sections were certified to be from the same liver and, for each purification, two 5µm sections were used (total of 10µm per purification). Five microliters of purified RNA were used as template in GoScript™ Reverse Transcription System per the manufacturer's instructions (Promega technical manual TM316) to generate complementary DNA. Five microliters of cDNA product were then used as template for multiplex PCR of the GAPDH gene (Promega GoTaq® Hot Start technical manual 9PIM500) whereby the four expected amplicons were 416, 290, 209, and 80bp long.

Results and discussions

To evaluate the amount of RNA purified from sequential 10µm sections of mouse liver tissue, RNA was purified from 3 individual sections per kit. The RNA was quantitated by

Table 1. RNA Quantitation Using A₂₆₀ Values.

	RNA Yields (ng/µl)						Kit Average
	Section 1	Section 2	Section 3	Section 4	Section 5	Section 6	
ReliaPrep™ FFPE Total RNA Miniprep System	765.35	675.20	745.17	N/A	N/A	N/A	728.57
Qiagen RNeasy FFPE Kit	N/A	N/A	N/A	839.47	696.79	836.52	790.93

Figure 1. Fragment Analysis Using Agilent Bioanalyzer. Agilent Bioanalyzer analysis of RNA purified from sequential 10µm FFPE sections of mouse liver tissue. Lane 1, Ladder, Lanes 2-4 ReliaPrep™ FFPE Total RNA Miniprep System products, Lanes 5-7 Qiagen RNeasy FFPE Kit products.

Figure 2. Fragmentation Analysis Using cDNA in Multiplex End-Point PCR. Gel image of multiplex end-point PCR products for the GAPDH gene. Lane 1, Ladder, Lanes 2-4 ReliaPrep™ FFPE Total RNA Miniprep System products, Lanes 5-7 Qiagen RNeasy FFPE Kit products.

determining the A_{260} value via NanoDrop. See Table 1 for results.

The apparent yields were higher with the RNeasy kit as opposed to the ReliaPrep™ FFPE Total RNA Miniprep method. To further evaluate the functional yield of the RNA prepared using these methods, aliquots of the prepared RNA were run on the Agilent Bioanalyzer. This analysis showed that more large-fragment RNA was obtained using the ReliaPrep™ FFPE Total RNA Miniprep System than with the Qiagen RNeasy FFPE Kit (Figure 1.)

To further analyze the functional yield of samples purified using these kits, multiplexed end-point PCR was performed on cDNA generated from RNA purified from sequential 10µm sections of human liver tissue. PCR products were run on a 1.5% agarose gel (Figure 2).

Fragments of 80, 209, and 290bp were detected by both kits. Bands for the 290bp fragment appear brighter for the ReliaPrep™ FFPE Total RNA Miniprep products. Neither kit could recover the 416bp fragment from this particular sample type.

Conclusion

These data indicate that determination of overall RNA yield by spectrophotometry alone is not sufficient in assessing RNA quality or suitability for downstream applications. Both kits purified RNA from each sample type, but the ReliaPrep™ FFPE Miniprep System purified more intact RNA from the mouse liver samples as evidenced by the Agilent Bioanalyzer analysis and as suggested in the band intensity in the human liver sample experiment. Obtaining longer fragments is useful when performing downstream analysis with precious samples.

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