Increasing total RNA yield from FFPE tissue samples using the microHomogenizer™ device

Introduction

Archived formalin-fixed, paraffin-embedded (FFPE) tissue samples offer a wealth of information on disease states related to cancer, infectious diseases, and toxicology. However, the quality of nucleic acids extracted from FFPE can vary significantly, depending upon how the tissues are prepared and the age of the sample. Therefore, extracting sufficient quality nucleic acid and protein from FFPE samples for downstream applications can be difficult and time intensive.

To address this issue, Denville Scientific offers the microHomogenizer™ device. Developed to rapidly homogenize fresh-frozen tissue in less than three minutes, the microHomogenizer™ device is also able to disaggregate FFPE core and section into smaller fragments allowing for improved RNA extraction without impacting RNA quality.

Materials and Methods

Formalin-Fixed Tissue: RNA was extracted from unsectioned core samples (15–20 mg) of a formalin-fixed human pancreatic cancer murine xenograft (BxPC3 cell line). The Qiagen RNeasy® FFPE kit was used to isolate total RNA as per manufacturer’s instructions. For the formalin-fixed samples, the deparaffinization step was excluded. microHomogenizer™ homogenization was included in the proteinase K digestion step and the sample was homogenized for three minutes.

FFPE Tissue: 10 mg core samples were taken from a two year old human liver FFPE block (Proteogenex Inc.). Total RNA was isolated using the Qiagen RNeasy® FFPE kit (n=3) and compared to the addition of a microHomogenizer™ device (n=5) processing step. FFPE samples were homogenized using microHomogenizer™ devices for two minutes in Qiagen’s deparaffinization solution prior to extraction. Extraction was carried out as per the manufacturer’s instructions.

Quantitative and Qualitative analysis of RNA: RNA concentrations were measured using a Tecan Infinite® M200 Pro Nanoquant. RNA quality was analyzed using the Bio-Rad Experion® system and RNA StdSens chip (Table 1).

RT-PCR analysis of RNA: cDNA was amplified using M-MLV reverse transcriptase (Promega) from 500ng of total RNA and primers targeting GAPDH or β-actin genes. Real-time PCR was performed using the ssoFast™ EvaGreen®Supermix (Bio-Rad) and 5µL of amplified cDNA as template. Cycling conditions were as follows: 98°C for 2 minutes (enzyme activation) followed by 98°C for 2 seconds to denature and 62°C for 4 seconds to anneal and extend (Repeated 44x).
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Figure 2. microHomogenizer™ (mH) increases total RNA yield from FFPE tissue samples. Total RNA isolated from FFPE human liver core sample using Qiagen’s FFPE RNeasy® kit (n=3) was compared to the addition of the mH processing step (n=5). RNA samples were analyzed on the Experion® system shown as a simulated gel (A) and electropherogram (B). (C-D) Total RNA yield (µg) and 260/280 ratios were quantified using an Infinite M200 Nanoquant. (E) RT-PCR amplification of GAPDH and β-actin using isolated RNA.

Figure 3. microHomogenizer™ device Improves Yield of Total RNA from Human Tissue FFPE samples. Comparison of RNA isolated from FFPE block core samples using Qiagen’s RNeasy® FFPE extraction kit +/- use of mH during deparaffinization. Total RNA yield was determined using a Nanoquant m200 Pro.

Results: Formalin fixed tissue: The addition of microHomogenizer™ device to the RNeasy® FFPE RNA isolation kit protocol increased total RNA yield from the formalin-fixed xenograft tissue by >4-fold (Table 1) versus the kit alone. In addition, the inclusion of microHomogenizer™ device in the purification protocol increased the RNA Quality Indicator (RQI) of the sample to 7.3 from 2.4 for the RNeasy kit alone (Figure 1).

FFPE Tissue: Based upon the age of the normal liver FFPE tissue sample (2 years) and the time of fixation (4 hours post mortem) it was expected to observe significant RNA degradation (Figure 2A-B) from the sample. As predicted, the total RNA exhibited considerable degradation; however, the sample prepared using microHomogenizer™ device showed a broader tailing indicating longer length RNA. Total RNA yield increased from 5.6 µg (+/- 1.3 µg) with the Qiagen kit alone, to 15.6 µg (+/- 4.4 µg) with microHomogenizer™ device (Figure 2C) without noticeable change in the A260/280 ratio (Figure 2D). RT-PCR analysis of the purified RNA showed that inclusion of microHomogenizer™ device did not impact RNA quality.

Conclusion: FFPE samples offer a significant challenge to researchers extracting nucleic acids for use in downstream applications. For biopsy core samples, which are often challenging to prepare, microHomogenizer™ device offers a simple and rapid method for extracting higher-yield RNA.

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