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Technical Note
PAXgene® Tissue System

Yield, purity, and integrity of RNA purified from PAXgene Tissue fixed, paraffin embedded (PFPE) rat tissue

Introduction

Tissue samples fixed and stabilized with the PAXgene tissue container can be processed and paraffin embedded for use in histopathological studies and purification of high-quality nucleic acids. Because this method does not exhibit the destructive cross-linking and degradation that results from formalin fixation, tissue morphology and nucleic acid integrity are preserved.

To prevent degradation during processing, a gentle, optimized processing protocol should be used: initial dehydration in 80% to 100% ethanol, fixation in low melting-point (≤54°C) paraffin, and incubation in liquid paraffin for no more than 3 hours. (For examples of gentle processing protocols, see the *PAXgene Tissue Container Product Circular*).

The PAXgene Tissue RNA Kit provides proven QIAGEN technologies to ensure RNA isolated from PAXgene Tissue fixed, paraffin embedded (PFPE) tissue is of maximum quality possible. RNA yield and integrity depend on several parameters in addition to the fixation chemistry and the processing protocol, including the type of tissue used, the period of cold ischemia (time from resection until fixation), the duration of fixation and stabilization, as well as the age and storage conditions of the paraffin embedded tissue. (The recommended storage temperature is –20°C; also see the technical note entitled *Morphology and RNA preservation in PAXgene Tissue fixed, paraffin-embedded tissue (PFPE) stored for 18 months at different temperatures*).

Purity, yield, and integrity of RNA purified from PFPE tissue prepared with the PAXgene Tissue RNA Kit were evaluated in this study for different rat (*Rattus norvegicus*) tissues: liver, kidney, spleen, intestine, and lung.

Note that, for clinical samples where parameters such as cold ischemia time are more difficult to control than when using an animal model, RNA integrity numbers (RIN) and yields may be lower than the values in this study.

Study Design

For each tissue type and parameter, the results from 58 sample preparations performed in 12 independent experiments were used to calculate median values for yield, purity, and integrity.

Tissue samples were cut into approximately 4 x 10 x 10 mm pieces and, within 5 minutes of resection, placed into standard tissue cassettes and attached to the cassette holder of a PAXgene Tissue Container for fixation (2–4 hours) and stabilization for up to 7 days at ambient temperatures or up to 6 months at –20°C or -80°C. Processing was performed on a Leica® TP1020 tissue processor according to recommendations in the *PAXgene Tissue Container Product Circular*.

After processing and embedding in paraffin, PFPE tissue was stored at 4°C or –20°C for up to 18 months. RNA was purified using the PAXgene Tissue RNA Kit from three to five 10 µm sections of PFPE tissue. RNA yield, purity, and integrity were determined.

Results

The median RNA yields from rat liver, kidney, spleen, intestine, and lung were 4.2, 2.2, 4.7, 4.7, and 0.9 µg RNA per 100 mm² tissue (Figure 1). This is consistent with results from several independent experiments that demonstrate that RNA yield varies depending on the tissue type.
RNA purity was fairly consistent for the different tissue types: median A_{260}/A_{280} values were 2.0–2.1 (Figure 2). Similar RIN median values were obtained for all tissue types: 7.2, 6.7, 7.3, 7.7, and 6.5 for liver, kidney, spleen, intestine, and lung, respectively (Figure 3).

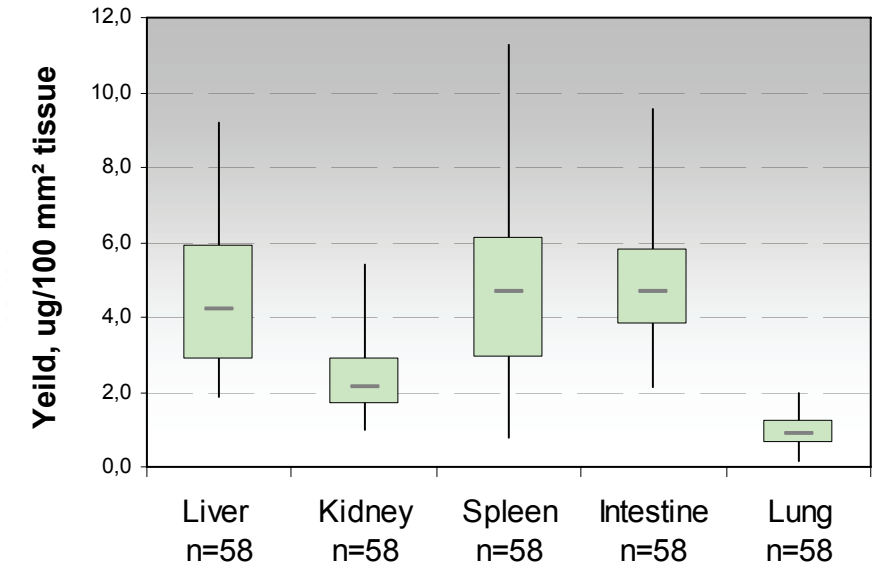

Figure 1. RNA yield. Samples were prepared and RNA was purified as described in "Study Design." Spectrophotometric analysis of RNA yield (absorbance at 260nm) was performed with a NanoDrop® Spectrophotometer. Yield was calculated as µg RNA per 100 mm² tissue. Box plots indicate median, lower, and upper quartile, as well as the minimum and maximum values observed.

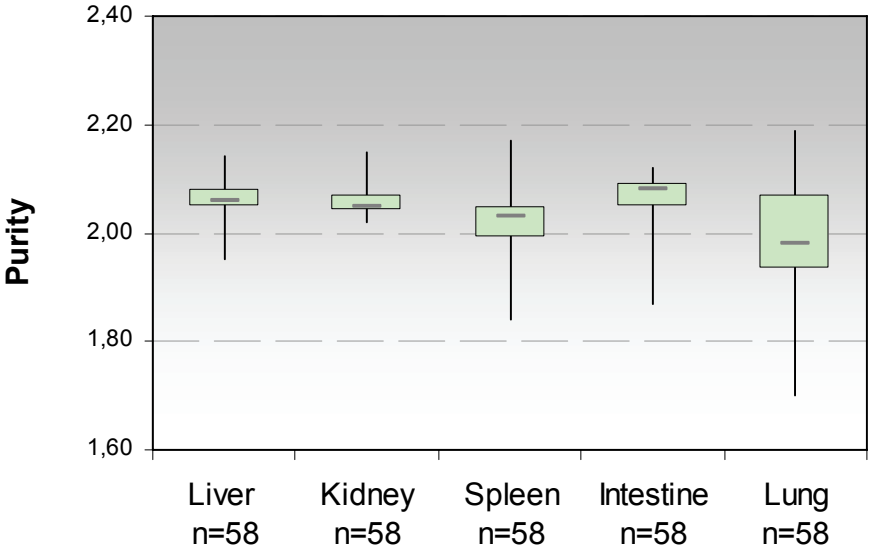

Figure 2. RNA purity. Samples were prepared and RNA was purified as described in "Study Design." Spectrophotometric analysis of RNA purity (A_{260}/A_{280} ratio) was performed with a NanoDrop Spectrophotometer. Box plots indicate median, lower, and upper quartile, as well as the minimum and maximum values observed.

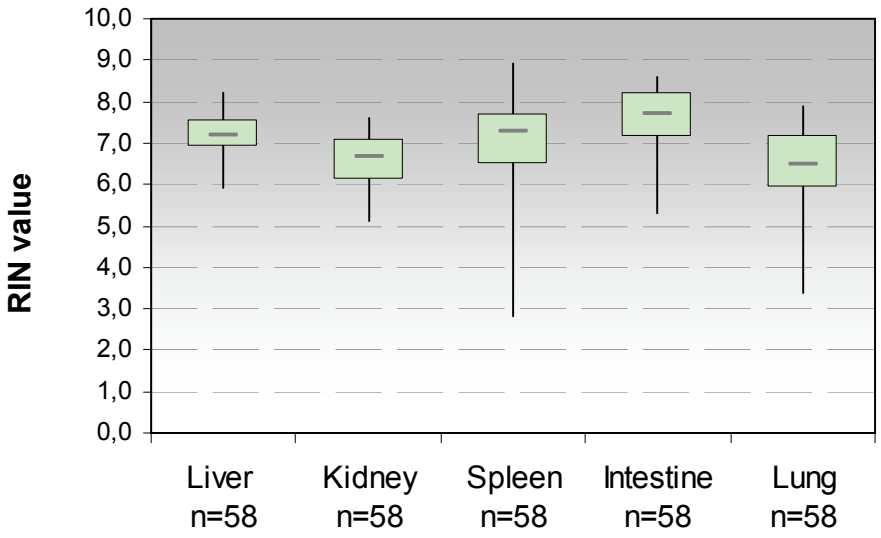

Figure 3. RNA integrity. Samples were prepared and RNA was purified as described in "Study Design." Electropherograms and RNA integrity numbers (RIN values) were obtained using the RNA 6000 Nano Chip Kit on an Agilent® Bioanalyzer. Box plots indicate median, lower and upper quartile, as well as the minimum and maximum values observed.

Conclusion

RNA with high purity and integrity can be isolated from sections of PFPE rat tissue that was fixed shortly after resection and processed according to the recommendation in the *PAXgene Tissue Container Product Circular*.

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