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Technical Note
PAXgene® Tissue System

Influence on RNA yield and integrity of modifications to the processing protocol for PAXgene Tissue fixed, paraffin embedded (PFPE) rat tissue

Study Design

Liver tissue from a single rat (*Rattus norvegicus*) was cut into approximately 4 x 10 x 10 mm pieces and placed into standard tissue cassettes. The cassettes were attached to the holder of PAXgene Tissue Container for fixation (1–3 hours) and stabilization (2–8 hours). As the standard protocol, processing was performed on a Leica® TP1020 tissue processor using the following recommendations from the *PAXgene Tissue Container Product Circular* (Table 1).

Table 1. Standard processing protocol

Step	Media	Time	Temperature	Vacuum
1	80% Ethanol	30 minutes	18–22°C	–
2	90% Ethanol	60 minutes	18–22°C	0.5 bar
3	95% Ethanol	60 minutes	18–22°C	0.5 bar
4	99% Ethanol	60 minutes	18–22°C	0.5 bar
5	99% Ethanol	60 minutes	18–22°C	0.5 bar
6	Isopropanol*	60 minutes	18–22°C	0.5 bar
7	Isopropanol*	60 minutes	18–22°C	0.5 bar

* Ethanol may be used instead of isopropanol.

Table 1. Standard processing protocol, continued

Step	Media	Time	Temperature	Vacuum
8	Xylene*	60 minutes	18–22°C	0.5 bar
9	Xylene*	60 minutes	18–22°C	0.5 bar
10	Paraplast X-tra®	30 minutes	56°C	0.5 bar
11	Paraplast X-tra	30 minutes	56°C	0.5 bar
12	Paraplast X-tra	60 minutes	56°C	0.5 bar

*Xylene substitutes may be used. Clearing agents based on D-limonene should not be used.

To determine the effects of modifying the standard processing protocol by changing the incubation temperature and/or duration of paraffin infiltration as well as by changing the ethanol concentration for the initial dehydration, modified processing protocols were tested (Table 2).

Table 2. Modifications to the standard processing protocol

Number	Step	Modification
1	10–12	Paraplast X-tra incubation at 60°C instead of 56°C
2	10–12	Paraplast X-tra incubation at 65°C instead of 56°C
3	10–12	Paraplast X-tra incubation at 70°C instead of 56°C
4	12	Paraplast X-tra incubation for 8 hours instead of 1 hour
5	1	Water incubation for 60 minutes instead of 80% ethanol for 30 minutes
6	1	40% ethanol incubation for 60 minutes instead of 80% ethanol for 30 minutes

After processing tissue and embedding in paraffin, RNA was purified using the PAXgene Tissue RNA Kit from four 10 µm sections of the PAXgene Tissue fixed, paraffin embedded (PFPE) tissue. All extractions were performed in triplicate. RNA yield and integrity were determined.

Results

The average RNA yield using the standard processing protocol was 11.3 µg. Increasing the temperature during incubation in liquid paraffin (modifications 1–3) had a moderately negative effect on RNA yield, with a 40% reduction of yield for

variant 3 (Figure 1). Prolonging the incubation time (modification 4) resulted in 60% yield reduction. The most dramatic impact on RNA yield was observed when the percentage ethanol in the initial dehydrating incubation was reduced: 86% and 87% yield reduction for modification 5 and 6, respectively (Figure 1).

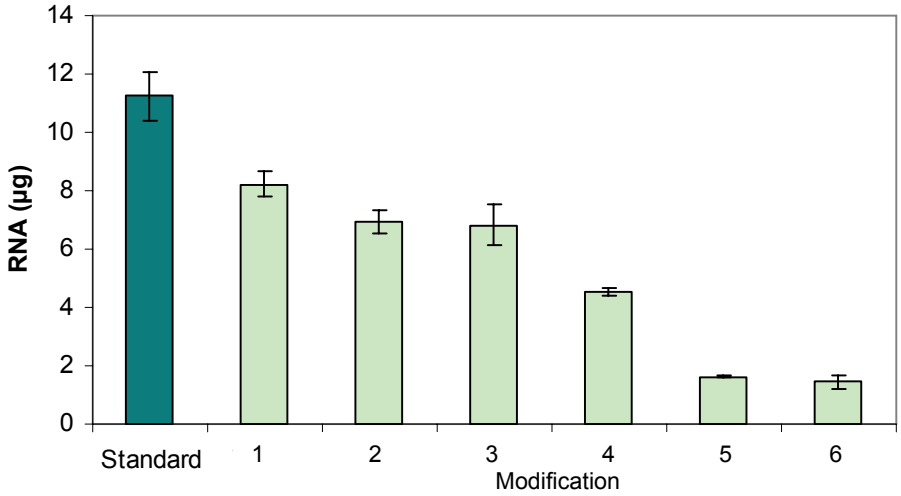

Figure 1. RNA yield. Samples were prepared and RNA was purified as described in "Study Design." Spectrophotometric analysis of RNA yield (absorbance at 260 nm) was performed with a NanoDrop® Spectrophotometer.

Modification of the processing protocol had little affect on RNA integrity: RNA integrity numbers (RIN) of 7.9–6.9 were observed for all protocols (Figure 2).

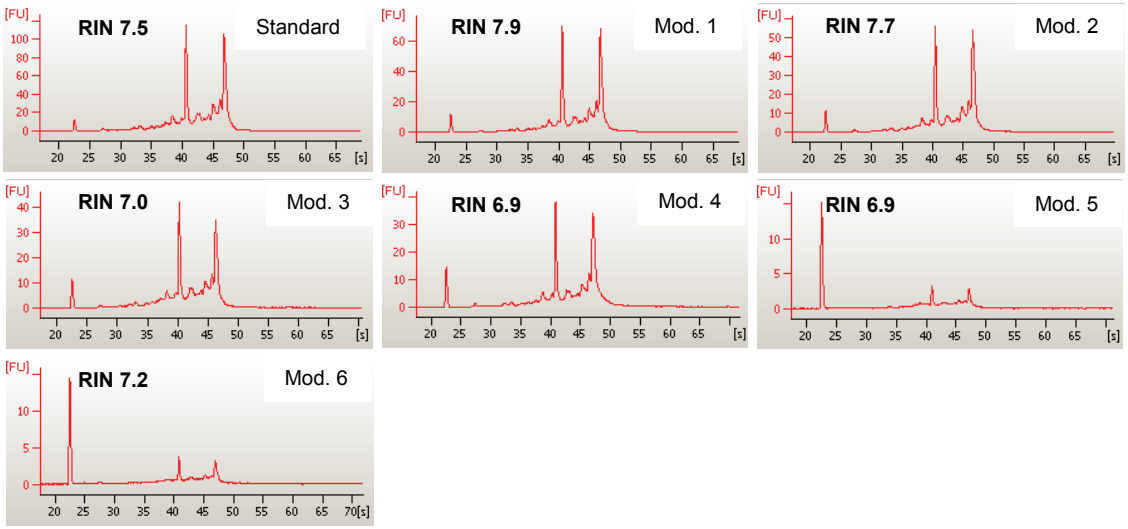

Figure 2. RNA integrity. Samples were prepared and RNA was purified as described in "Study Design." Electropherograms and RNA integrity numbers (RIN values) were obtained using the RNA 6000 Nano Chip Kit on an Agilent® Bioanalyzer.

Conclusion

When working with tissue fixed with the PAXgene Tissue Container, the following modifications to the standard processing protocol can result in drastically reduced RNA yields: paraffin incubation periods longer than 3 hours, paraffin incubation temperatures higher than 60°C, and reduction of the ethanol content for the initial dehydration.

When processing specimens fixed in PAXgene Tissue Containers, follow processing protocol recommendations in the *PAXgene Tissue Container Product Circular*. In particular, be sure to use 80–100% ethanol for the initial dehydration and low melting-point (≤ 54°C) paraffin and do not incubate in liquid paraffin for longer than 3 hours.

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