PreAnalytiX
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Technical Note
PAXgene® Tissue System

Morphology and RNA preservation in PAXgene Tissue-fixed, paraffin-embedded tissue (PFPE) stored for 18 months at different temperatures

The PAXgene Tissue Container and the PAXgene Tissue RNA Kit, products used in the methods of this technical note, have been discontinued. We recommend using the PAXgene Tissue FIX Container (Cat. no. 765312), PAXgene Tissue STABILIZER (Cat. no. 765512) and the PAXgene Tissue RNA/miRNA Kit (Cat. no. 766134) as alternative solutions.

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Study Design

Rat *(Rattus norvegicus)* tissue from liver, kidney, spleen, lung and intestine was grossed into samples of approximately 4 x 10 x 10 mm size. Samples were placed into standard histocasettes for fixation (4 hours) with PAXgene Tissue FIX then transferred into PAXgene Tissue STABILIZER and incubated for an additional 20 hours. Samples were then processed and paraffin embedded (PFPE) following the recommendations in the *PAXgene Tissue Container Product Circular*. As references, mirrored samples from all tissue types (4 x 10 x 10 mm) were fixed for 24 hours in neutral buffered formalin, processed and paraffin embedded (FFPE). In addition, samples (4 x 10 x 10 mm) from all tissue types were snap frozen in liquid nitrogen (LN_2) and 10 mg of tissue was used for RNA extraction with the RNeasy® Mini Kit. This RNA was used as reference and stored at –20°C until use.

PFPE and FFPE samples were stored at 22°C, 4°C and –20°C. After 18 months storage, 4 µm sections were stained with hematoxylin and eosin (H&E) for analysis of preservation of the morphology. RNA was purified from 3 sections with 10 µm thickness each of PFPE with the PAXgene Tissue RNA Kit and from FFPE with the RNeasy FFPE Kit.

RNA yield was analyzed by measuring the absorbance at 260 nm. Performance in real-time RT-PCR was analyzed with a TaqMan® Primer/Probe assay for β-actin. End-point one-step RT-PCR was performed with sequences of increasing length for the rat hypoxanthine phosphoribosyl transferase (HPRT) mRNA.

Results

After 18 months storage at 22°C, blocks of PFPE rat tissue were intact and could easily be sectioned into 4 µm thin sections for H&E staining. The morphology of H&E stained sections was intact without artifacts as a result of the storage period (Figure 1, PFPE storage at 22°C). Intact blocks of tissue and morphology free of artifacts were also observed in PFPE tissue stored at 4°C and –20°C (data not shown).

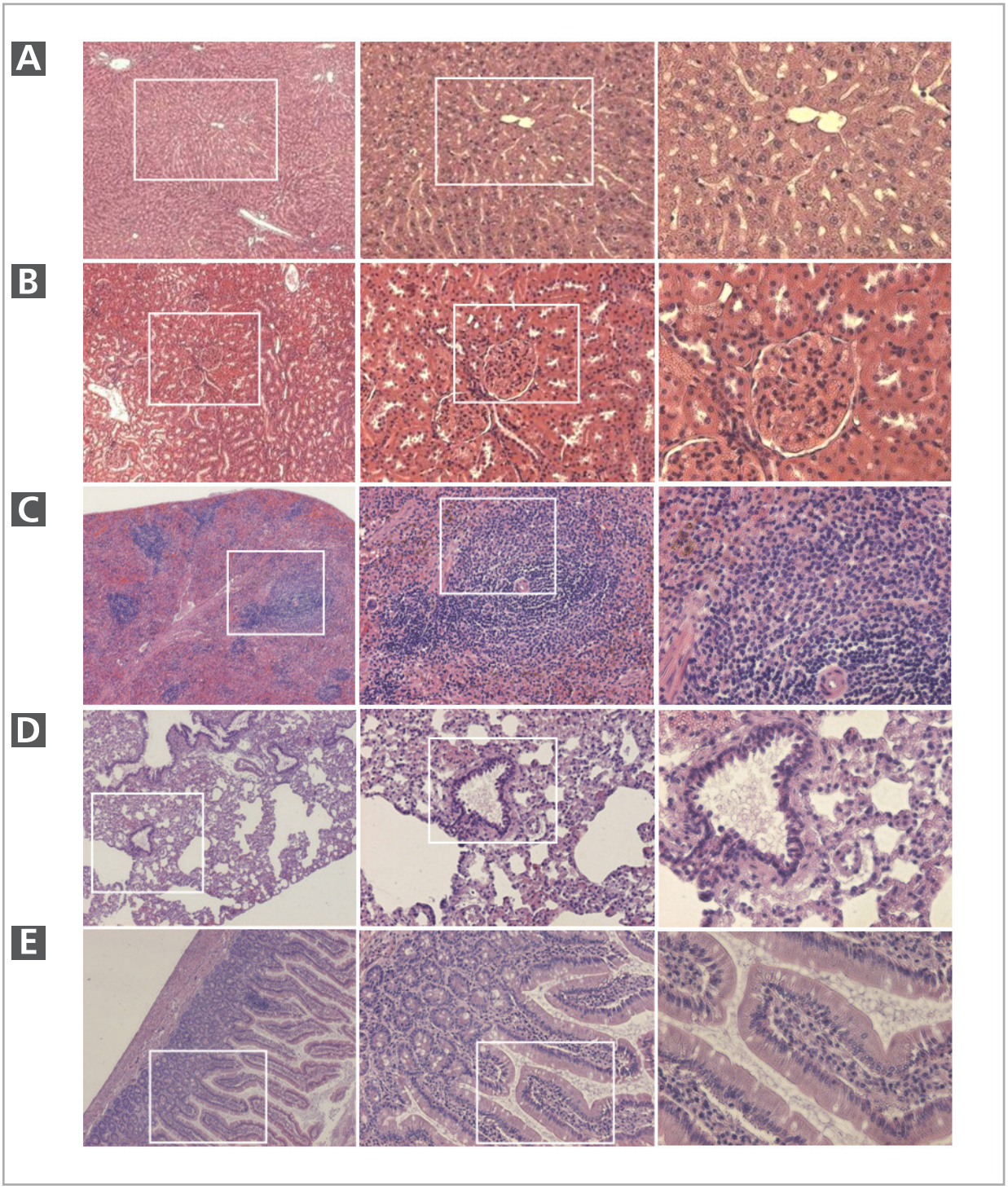

Figure 1. Preserved morphology after 18 months storage of PFPE (PAXgene Tissue-fixed paraffin-embedded) tissue at 22°C. Hematoxylin and eosin staining of 4 µm sections of rat tissue PFPE, stored for 18 months at 22°C. **A** liver, **B** kidney, **C** spleen, **D** lung, **E** intestine; 40x, 100x and 200x magnifications.

RNA could be isolated with the PAXgene Tissue RNA Kit. RNA yield from 3 sections with a thickness of 10 µm varied according to the tissue type between 1 µg (lung) and up to 10 µg (liver). Storage temperature of PFPE seemed to have a minor influence on RNA yield (**Figure 2**).

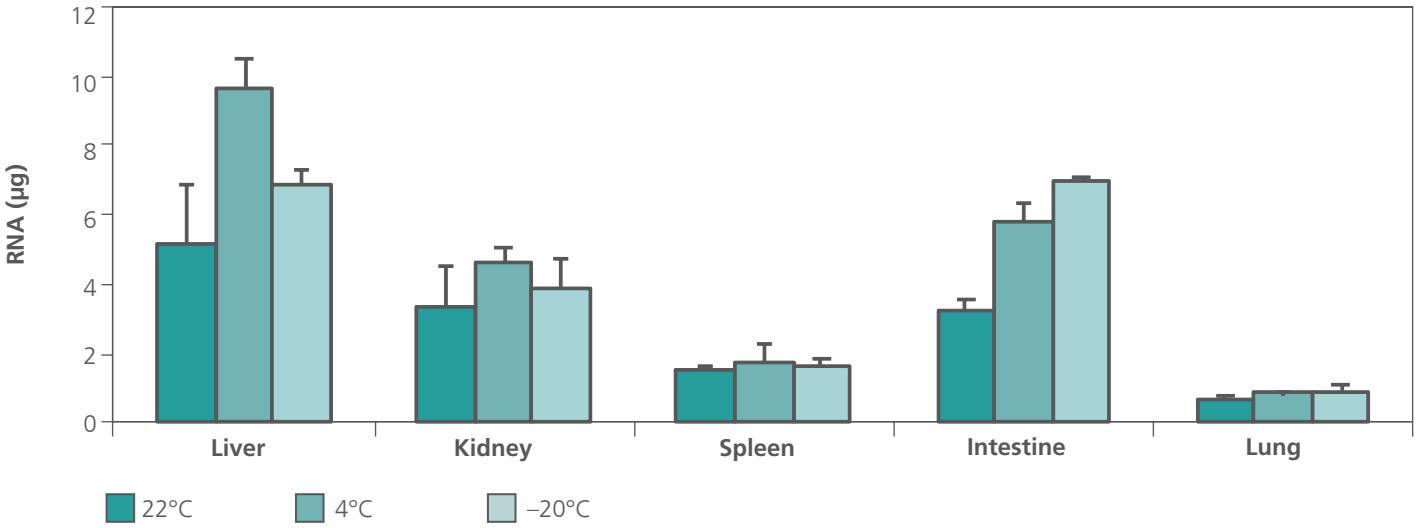

Figure 2. RNA yield from 3 x 10 µm sections of PFPE after storage for 18 months at different temperatures. Spectrophometric analysis of RNA yield with a NanoDrop® instrument. RNA was purified from PFPE with the PAXgene Tissue RNA Kit after 18 months storage at 22°C, 4°C or –20°C.

RNA isolated from PFPE and FFPE was tested for performance in real-time RT PCR using a primer/probe assay for amplification of a 294 bp fragment of β-actin mRNA. C_T values were compared to the C_T values from RNA purified from tissues snap frozen in LN_2. In the case of RNA from FFPE tissue, regardless of the storage temperature, the ΔC_T values of RNA from frozen tissue ranged between 7 and 11, reflecting the inhibition of the RT-PCR due to chemical modifications of the RNA introduced by formalin. In the case of RNA from PFPE the ΔC_T values of RNA from frozen tissue depended on storage temperature. Storage at 4°C or –20°C resulted in ΔC_Ts between 1 and –1, which means no significant difference, but storage at 22°C resulted in an average ΔC_T of 2 for liver, kidney, spleen and intestine and 4 in the case of lung (**Figure 3**).

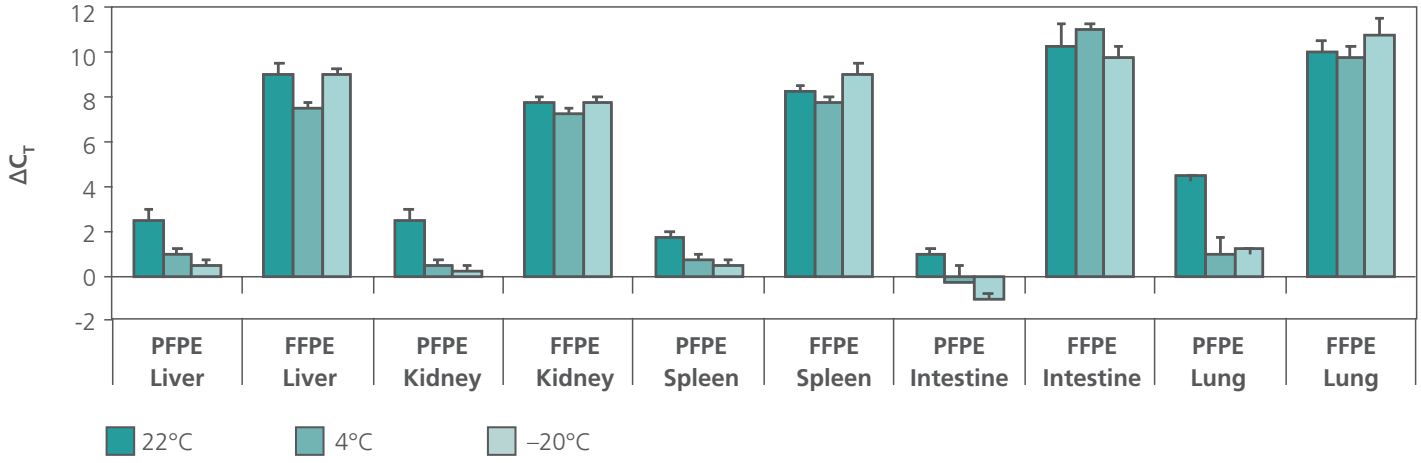

Figure 3. β-Actin real-time RT-PCR with RNA from PFPE or FFPE after storage for 18 months at different temperatures. Gene expression analysis of β-actin by quantitative real-time RT-PCR. Triplicate RNA extraction from PFPE and FFPE tissue stored for 18 months at 22°C, 4°C or –20°C were amplified in duplicate using 10 ng RNA with the QIAGEN® QuantiTect® Probe RT-PCR Kit and compared to assay results with reference RNA from tissues snap frozen in liquid nitrogen (LN_2); $\Delta C_T = C_T$(RNA from PFPE or FFPE) – C_T(RNA from LN_2).

In end-point RT-PCR using 10 ng of RNA, a 668 bp fragment could be amplified, regardless of the storage temperature of the PFPE tissue blocks (**Figure 4**). A 1065 bp fragment could be amplified from all PFPE blocks of tissue stored at 4°C or –20°C, but could not be amplified from PFPE tissue stored at 22°C. In contrast, regardless of the storage conditions, RNA from FFPE blocks of tissue produced a reliable positive result only in case of the 128 bp fragment. Amplification of the 404 bp fragment sometimes produced a weak band (**Figure 4**).

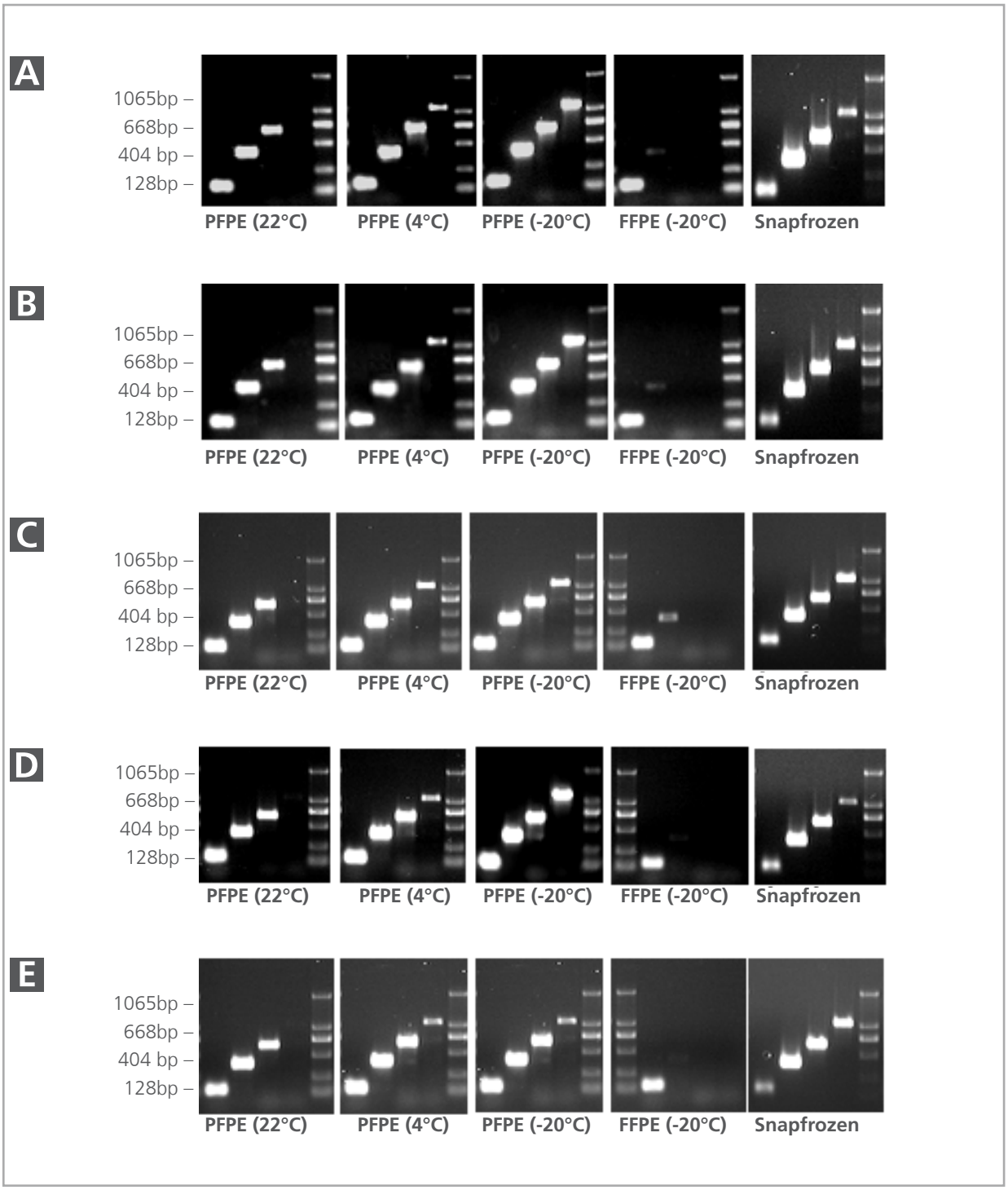

Figure 4. End-point RT-PCR of gene fragments up to 1 kb with RNA from PFPE or FFPE after storage for 18 months at different temperatures. One-step, end-point RT-PCR of 128, 404, 668 and 1065 bp sequences of the rat hypoxanthine phosphoribosyl transferase (HPRT) mRNA with RNA from **A** rat liver, **B** kidney, **C** spleen, **D** intestine and **E** lung. The RNA was isolated from PFPE or FFPE tissue stored for 18 months at 22°C, 4°C or –20°C, as well as from tissue snap frozen in LN_2. Amplification was performed with 10 ng RNA using the QIAGEN OneStep RT-PCR Kit.

Conclusion

PFPE blocks of tissue can be stored at –20, 4 or 22°C for up to 18 months without damage to the morphology. The RNA isolated from the PFPE tissues can be used in RT-PCR for amplification of mRNA sequences up to 668 bp in length. However for optimal preservation of RNA, storage at –20°C is recommended.

Studies on long-term storage of FFPE and PFPE tissues are ongoing. RNA and DNA have been extracted from stored tissue samples at several intervals up to 9 years and examined for integrity and usability in quantitative RT-PCR (RT-qPCR) or PCR (qPCR) assays.

See the following publication:
Groelz, D., Viertler, C., Pabst, D., Dettmann, N. and Zatloukal, K. 2018. Impact of storage conditions on the quality of nucleic acids in paraffin embedded tissues. PLoS One 13, e0203608.

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