RT-PCR Performance of RNA Obtained From Archived Formalin or PAXgene® Tissue Fixed, Paraffin-embedded (FFPE and PFPE) Blocks of Tissue

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Introduction

PAXgene Tissue is a non-crosslinking, alcoholic tissue fixation and stabilization system. Sections of PAXgene Tissue fixed, paraffin-embedded (PFPE) tissue can be used for histological staining or purification of high quality DNA, RNA, miRNA, and proteins.

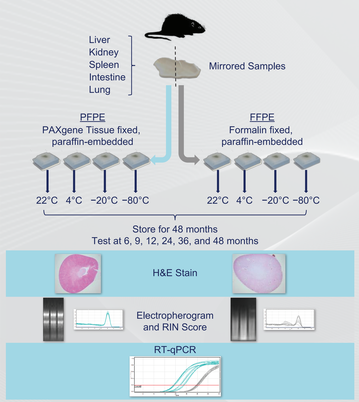
In a long-term storage study, mirrored samples of PFPE and FFPE rat tissues were stored at different temperatures, and RNA was extracted at regular intervals for subsequent analysis of integrity and performance in quantitative (q), real-time RT-PCR.

Here we present RNA integrity and RT-qPCR results from PFPE and FFPE tissues stored for up to 48 months at different temperatures.

Methods

Study Design:

1. Rat intestine, spleen, kidney, lung, and liver were divided into mirrored sample pairs, placed into histocassette and fixed at room temperature in either the PAXgene Tissue Container (PreAnalytiX) or with neutral buffered formalin (Sigma).
2. Fixed tissues were processed and embedded in paraffin blocks which were stored at 22°C, 4°C, –20°C, or –80°C.
3. At the beginning of the study, and after 6, 9, 12, 24, 36, and 48 months, RNA was extracted from sections of PFPE and FFPE tissue and additional sections were stained with hematoxylin and eosin (H&E).
4. RNA was analyzed for integrity, yield, and purity. RT-qPCR assays with a variety of amplicon lengths were used to evaluate analytical performance of the PFPE and FFPE RNA in comparison to RNA from snap-frozen tissue.

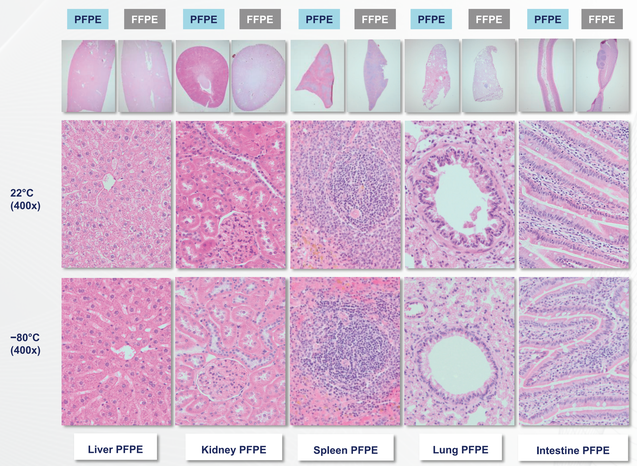


RNA extraction: PAXgene Tissue RNA Kit (PreAnalytiX) (PFPE); RNeasy® FFPE Kit (QIAGEN) (FFPE); RNeasy Mini Kit (QIAGEN) (Snap-frozen tissue).

RT-qPCR: One-step RT-PCR with QuantiTect® Probe or QuantiTect SYBR® Green RT-PCR Kits (QIAGEN).

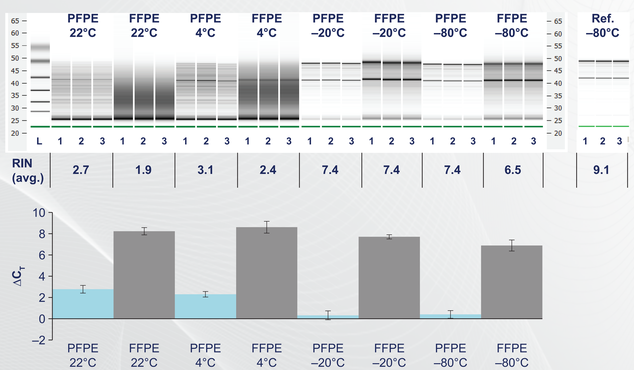
Results

H&E stained sections of PFPE and FFPE rat tissue stored for 48 months



- H&E histology is preserved in all PFPE samples stored between 22°C to –80°C for up to 48 months.
- Storage of PFPE blocks at –80°C led to cracks within the paraffin which did not affect sectioning or histological structure of the tissue.

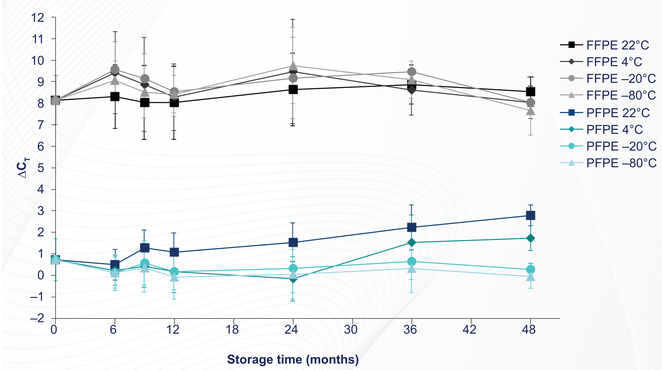
Integrity and performance in RT-qPCR of RNA from PFPE and FFPE rat tissue stored for 48 months



- Rat liver RNA PFPE & FFPE stored 48 months / RT-qPCR 10 ng RNA
- RIN scores of RNA from FFPE tissue do not reflect chemical modifications of RNA. FFPE tissue RNA exhibits severe RT-qPCR inhibition.
- RIN scores of RNA from PFPE tissue are higher than or equal to those of RNA from FFPE tissue at each temperature studied, but PFPE tissue RNA does not inhibit RT-qPCR.

Note: Average ΔC_T values are shown for triplicate extractions amplified in duplicate ($\Delta C_T = C_T[FFPE] - C_T[Ref.]$ or $\Delta C_T = C_T[PFPE] - C_T[Ref.]$).

Performance differences in RT-qPCR between PFPE and FFPE tissue RNA from blocks stored for 48 months at four different temperatures

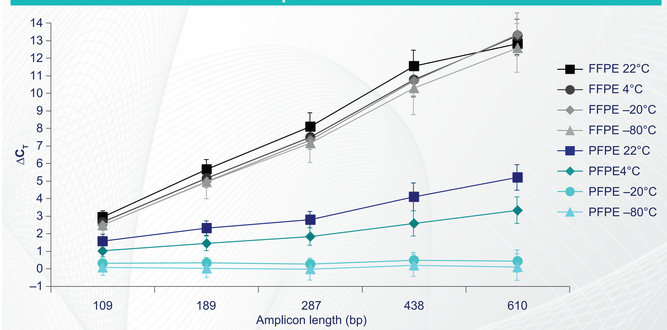


- ΔC_T of PFPE tissue RNA is 0–3 compared to 7.5–10 for FFPE tissue RNA extracted from blocks stored for 48 months at four different temperatures.
- RNA from PFPE performed better than RNA from FFPE samples regardless of storage time (up to 48 months) or temperature (–80°C to 22°C). This was despite the fact that RIN scores for RNA extracted from PFPE and FFPE were comparable.

Note: Reference (Ref.) tissue was snap-frozen in liquid nitrogen and stored at –80°C.

RT-PCR assay of the rat β-actin gene (amplicon length: 294 nt.). Average ΔC_T values ($\Delta C_T = C_T[FFPE] - C_T[Ref.]$ or $\Delta C_T = C_T[PFPE] - C_T[Ref.]$) are shown for triplicate extractions from five different types of tissue (liver, kidney, spleen, lung, and intestine) amplified in duplicate for each fixation method, storage temperature, and time point of extraction.

SYBR Green real-time RT-qPCR* of RNA from PFPE and FFPE blocks stored for 48 months at different temperatures

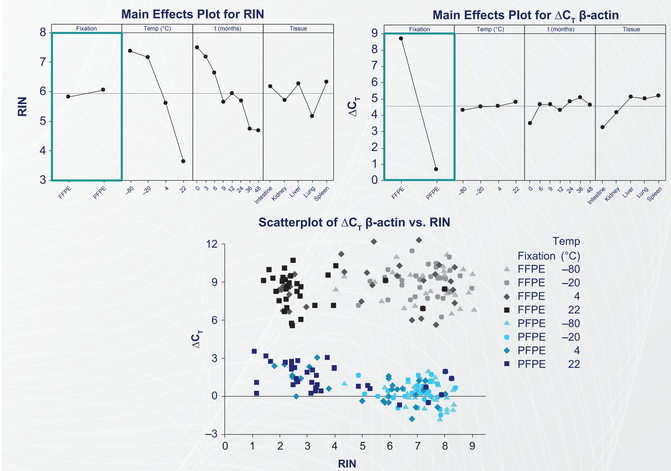


- Performance differences in RT-qPCR between PFPE and FFPE tissue RNA from blocks stored for 48 months increases with amplicon length.
- Compared to RNA from snap-frozen tissue, RNA from FFPE tissue showed amplicon length-dependent inhibition. In contrast, RNA from frozen PFPE blocks with high RIN values performed as well as RNA from snap-frozen tissue regardless of amplicon length.
- RNA with lower RIN values from PFPE blocks stored at ambient temperatures showed slightly increased C_T values as compared to snap-frozen tissue.

*Five different SYBR Green real-time, one-step RT-PCR assays of rat β-actin gene with one common forward primer and five different reverse primers to generate amplicon lengths from 109 to 610 base pairs.

Average ΔC_T values ($\Delta C_T = C_T[FFPE] - C_T[Ref.]$ or $\Delta C_T = C_T[PFPE] - C_T[Ref.]$) are shown for triplicate extractions from five different tissue types (liver, kidney, spleen, lung, and intestine) amplified in duplicate for each fixation method and storage temperature.

Main effects and scatter plots of ΔC_T values and RIN scores for FFPE and PFPE tissue stored for 48 months at 4 different temperatures



- RNA degraded over time independent of the fixation method but dependent upon storage temperature. Degradation was slowed at 4°C and stopped when blocks were stored frozen.
- RIN score is not a predictor of FFPE tissue RNA performance in RT-qPCR.
- Main effects plots show that the mode of fixation has the highest impact on ΔC_T value.

Conclusions

- Morphology is preserved in archived PFPE tissue.
- RNA degrades in paraffin embedded blocks of tissue. The rate of degradation depends on storage temperature.
- Advantages in quantitative, real-time RT-PCR persist for RNA from archived PFPE versus FFPE tissue, regardless of storage temperature.
- RIN scores of RNA from FFPE samples are not a positive predictor of performance in RT-PCR.

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