Stability of Nucleic Acids in Archived Formalin and PAXgene® Tissue Fixed, Paraffin-embedded (FFPE and PFPE) Blocks of Tissue

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Objective

To evaluate morphology and nucleic acids in PFPE and FFPE tissue, stored for four years at different temperatures.

Methods

- Tissue fixation, processing, and storage
  - Rat liver, kidney, spleen, lung and intestine were grossed immediately after resection. From each organ, mirrored pairs of samples, identical in size, were placed into tissue cassettes and fixed at room temperature in either the PAXgene Tissue Container (PreAnalytiX) or with neutral buffered formalin (Sigma).
  - PAXgene: 4 h fixation, 24 h stabilization
  - Formalin: 24 h fixation
  - All samples were processed on a TP1020 with 70%, 80%, 90%, 99% (2x) ethanol, isopropanol (2x), xylene (2x), paraffin (3x, Paraplast-XTRA) at 56℃
  - Replicates of PAXgene Tissue fixed, paraffin embedded (PFPE) and Formalin fixed, paraffin embedded (FFPE) blocks were stored for four years at 22℃, 4℃, -20 ℃ or -80℃.
- RNA extraction and analysis
  - RNA was extracted from 3 x 10 µm sections of PFPE tissue with PAXgene Tissue RNA kit (PreAnalytiX) and from FFPE tissue with RNeasy® FFPE kit (QIAGEN) and eluted in 30 µl elution buffer supplied in the kits.
  - Yield was determined with a Nanodrop Spectro-photometer and integrity with an Agilent Bioanalyzer.
  - Real time RT-PCR was conducted with a Rotor-Gene Q (QIAGEN) in one-step RT-PCR reactions using the QuantiTect SYBR® Green RT-PCR kit (QIAGEN) with assay volumes of 25 µl with 2 µl eluates diluted 1:10 with water.
- DNA extraction and analysis
  - DNA was extracted from 3 x 10µm sections of PFPE with PAXgene Tissue DNA kit (PreAnalytiX) and from FFPE with QIAamp® FFPE kit (QIAGEN) and eluted in 30 µl elution buffer supplied in the kits.
  - Yield was determined with a Nanodrop Spectro-photometer and integrity on agarose gel electrophoresis.
  - Real time PCR was conducted with a Rotor-Gene® Q (QIAGEN) in a one-step PCR reactions using the QuantiTect SYBR® Green PCR kit (QIAGEN) with assay volumes of 25 µl with 2 µl eluates diluted 1:10 with water.

Results

- Nucleic acid degradation occurs in FFPE and PFPE tissue at ambient temperatures (Fig. 1 and 4).
- No nucleic acid degradation was observed in frozen FFPE and PFPE tissue (Fig. 1 and 4).
- There were advantages in PCR performance with regard to lower C_T values and less failures for RNA and DNA from PFPE versus FFPE samples regardless of storage temperature (Fig. 2 and 5).
- RNA and DNA from frozen FFPE samples show advantages in PCR as compared to RNA and DNA from samples stored at ambient temperatures (Fig. 3 and 5C).
- H&E histology was preserved in PFPE samples stored for up to four years (Fig. 6 – data shown for storage at 22℃ only).

Conclusion

- After storage for four years, tissue morphology is preserved in archived PFPE tissue.
- After storage for four years, nucleic acids from PFPE samples perform better in PCR and RT-PCR than nucleic acids from FFPE samples.

RNA Integrity and RT-PCR Performance

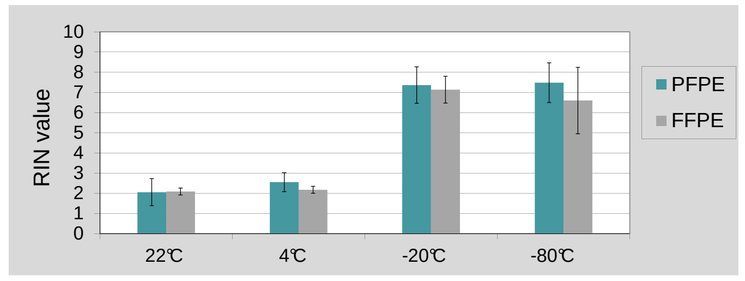


Fig. 1: RNA integrity values for RNA isolated from rat PFPE and FFPE tissue stored for 4 years at 22℃, 4℃, -20℃ or -80℃. Average values from liver, kidney, spleen, lung and intestine with three replicates each.

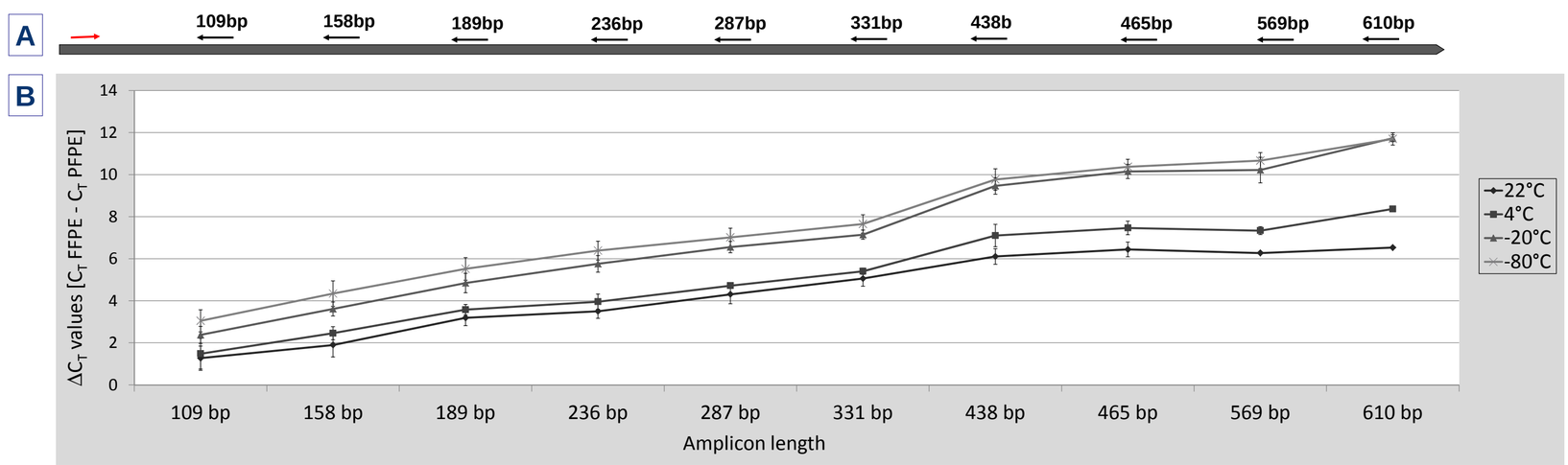


Fig. 2: Performance differences in SYBR Green real time RT-PCR between RNA from PFPE and FFPE tissue. A: Assay design for amplification of rat beta-actin gene with one common forward primer and different reverse primers to generate amplicon lengths from 109 to 610 base pair. B: ΔC_T values calculated by subtraction of C_T values obtained with RNA from PFPE blocks stored for four years at 22℃, 4℃, -20℃ or -80℃ from C_T values obtained with RNA from corresponding FFPE samples; average values and standard deviations from triplicate extractions of five different tissue types. C: Example for amplification plot and melting curve analysis from rat liver PFPE and FFPE tissue.

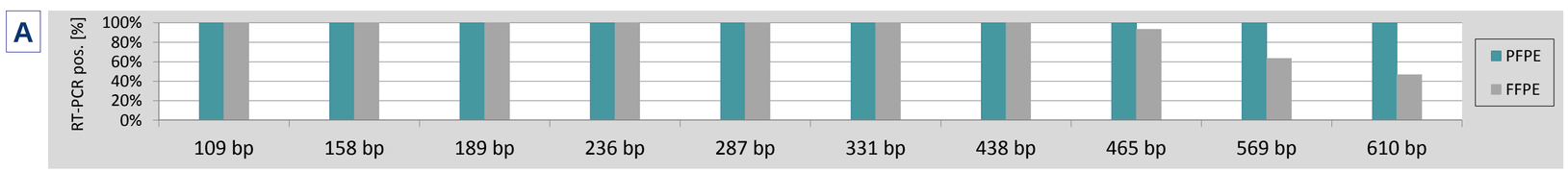
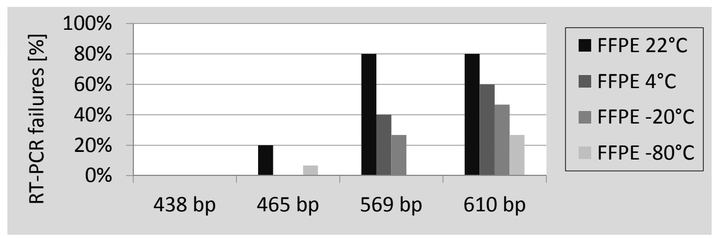


Fig. 3: Performance in SYBR Green real time RT-PCR. A: Number of samples [%] positive for each assay from Fig. 2. B: Detailed analysis of PCR failures [%] for FFPE samples stored at 22℃, 4℃, -20℃ or -80℃.



DNA Integrity and PCR Performance

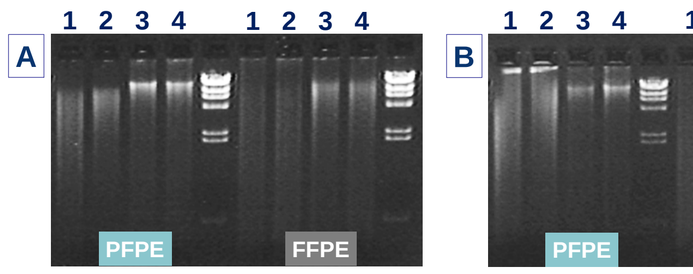


Fig. 4: DNA integrity analysis from PFPE and FFPE tissue by agarose gel electrophoresis. PFPE and FFPE blocks were stored prior to DNA extraction for 4 years at 22℃ (1), 4℃ (2), -20℃ (3) and -80℃ (4). 200 ng genomic DNA was separated on 0.8% TAE agarose gels. Examples are shown for DNA from rat kidney (A) and intestine (B).

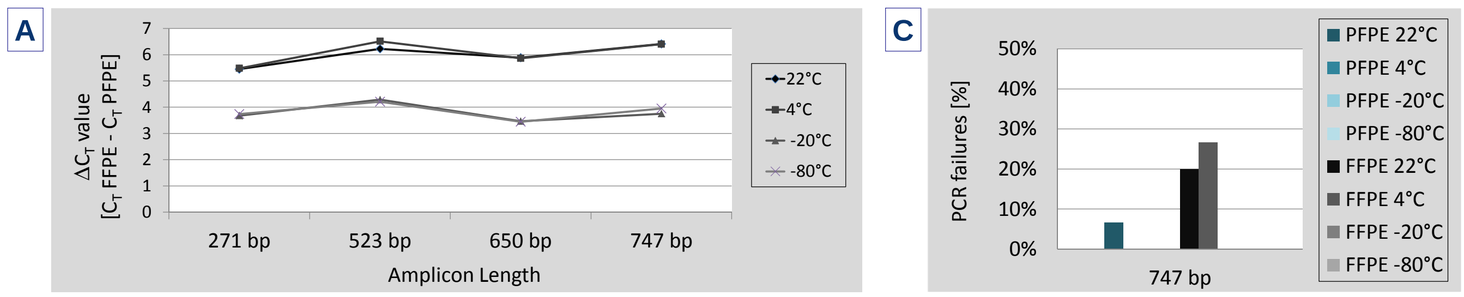


Fig. 5: Performance differences between DNA from PFPE and FFPE tissue in four different SYBR Green real time PCR assays for amplification of 271, 523, 650 or 747 bp of rat beta-actin gene. A: ΔC_T values calculated by subtraction of C_T values obtained with DNA from PFPE blocks stored for 4 years at 22℃, 4℃, -20℃ or -80℃ from C_T values obtained with DNA from corresponding FFPE samples; average values and standard deviations from triplicate extractions of five different tissue types. B: Example for amplification plot and melting curve analysis from rat spleen PFPE and FFPE tissue. C: Number of samples [%] stored at different temperatures negative for the 747 bp assay.

H&E Morphology Preservation

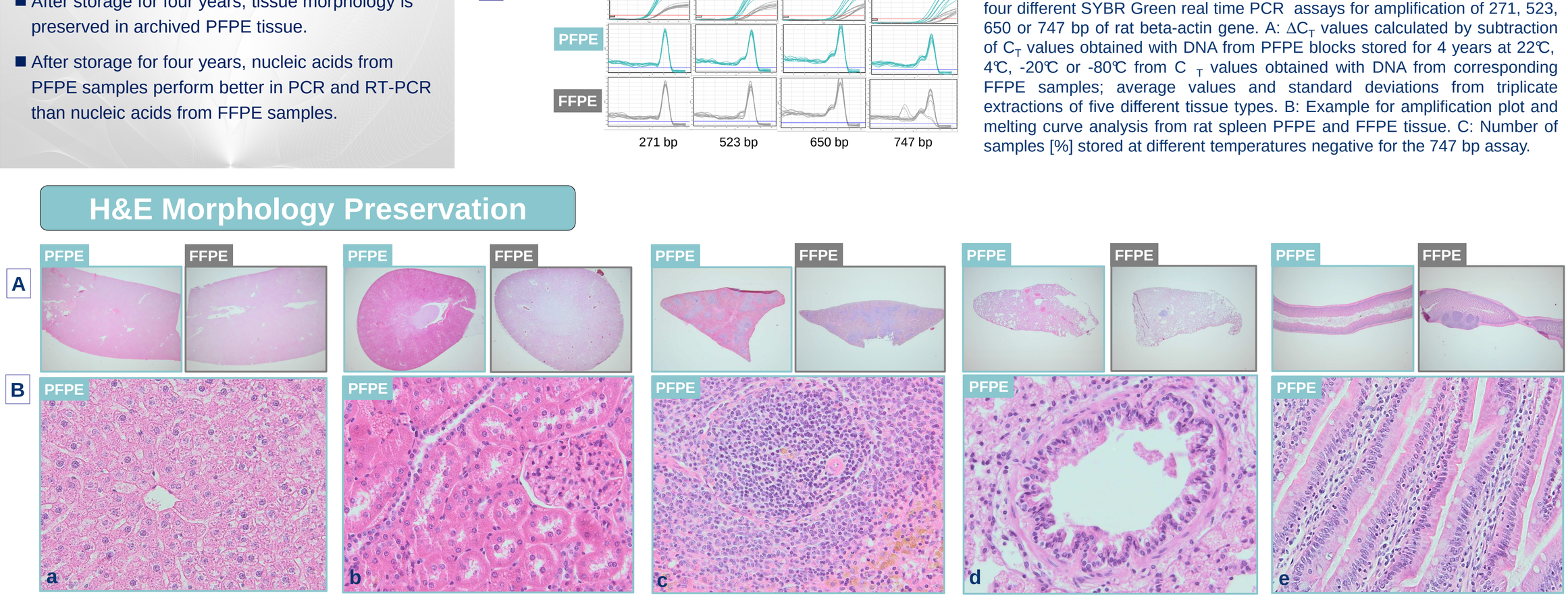


Fig. 6: Hematoxylin & Eosin stained sections of FFPE and PFPE blocks of rat tissue stored for 4 years at 22℃.
A: Overview of whole section with identical sizes for PFPE and FFPE samples from each organ. B: Morphology of rat liver (a), kidney (b), spleen (c), lung (d) and intestine (e); original magnification x400.