PAXgene® Tissue Fixation Technology for Simultaneous Preservation of Morphology and Biomolecules

SPIDIA: Standardization and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics

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Background

Current methods for tissue preservation involve fixation with formalin or snap-freezing in liquid nitrogen. Formalin preserves tissue by cross-linking proteins and nucleic acids. This cross-linking causes irreversible chemical modification and damage to protein and nucleic acid biomarkers, particularly RNA. While cryopreservation of tissue is the gold standard for biomolecule preservation, histomorphology in frozen tissue is usually compromised, sectioning of unfixed, frozen samples is technically demanding, and special equipment is required.

PAXgene Tissue (PAXgene) is a new, formalin-free fixation technology. PAXgene fixed, paraffin embedded (PFPE) tissue is suitable for conventional histochemical[1] and immunohistochemical staining as well as for extraction of high quality nucleic acids[2] and proteins including phosphoproteins.[3]

Material & Methods

Cases of human cancer were divided and fixed either for 24 hours in neutral buffered formalin, for 2-24 hours in PAXgene Tissue fixative, followed by stabilization of up to 4 days in PAXgene Tissue Stabilizer, or snap frozen in liquid nitrogen (LN2). After processing and paraffin-embedding FFPE and PFPE tissue were stained with H&E and IHC stains. Nucleic acids were isolated and analyzed in RT-qPCR, by agarose gel electrophoresis, long-range, and multiplex PCR (Tab.1). Proteins and phosphoproteins were evaluated by western blot analysis.

Table 1. Nucleic acids preparation and amplification kits and antibodies used

	Snap frozen (LN2)	PFPE	FFPE
Nucleic acid purification kits	RNeasy® Mini, miRNeasy Mini, QIAamp® Mini (QIAGEN)	PAXgene Tissue RNA, miRNA, DNA (PreAnalytiX)	RNeasy FFPE, miRNeasy FFPE, QIAamp FFPE (QIAGEN); RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion)
PCR	QuantiTect® Probe PCR, QIAGEN® LongRange and QuantiTect Multiplex PCR Kits (QIAGEN)		
PCR arrays	TaqMan® Array Gene Signature 96-Well Plates (Life Technolgies) RT² Profiler™ PCR Array Human miFinder (SABiosciences)		
Antibodies for IHC	anti CK20 clone K20.8, anti Ki-67 clone MIB-1, anti ERα clone 1D5 (Dako), anti PR clone 1A6 (Bioprime)		
Antibodies for Western Blotting	beta-Actin (Sigma-Aldrich), GAPDH (Stressgen Biomol), Phospho-PRAS40, -p44/42 MAPK, and -p38 MAPK (Cell Signaling Technolgy)		

Results

Morphology of PFPE samples and IHC staining intensities were similar to that for FFPE tissue (Fig.1). High correlation of RNA and miRNA expression between PFPE and LN2 samples, but poor correlation between FFPE and LN2 was observed (Figs. 2 and 3). DNA from LN2 and PFPE was of high molecular weight and performed well in PCR, but DNA from FFPE failed in most PCR assays (Fig. 4). Signal intensities for phosphoproteins in western blots were comparable between LN2 and PFPE, but weaker for FFPE tissue (Fig. 5).

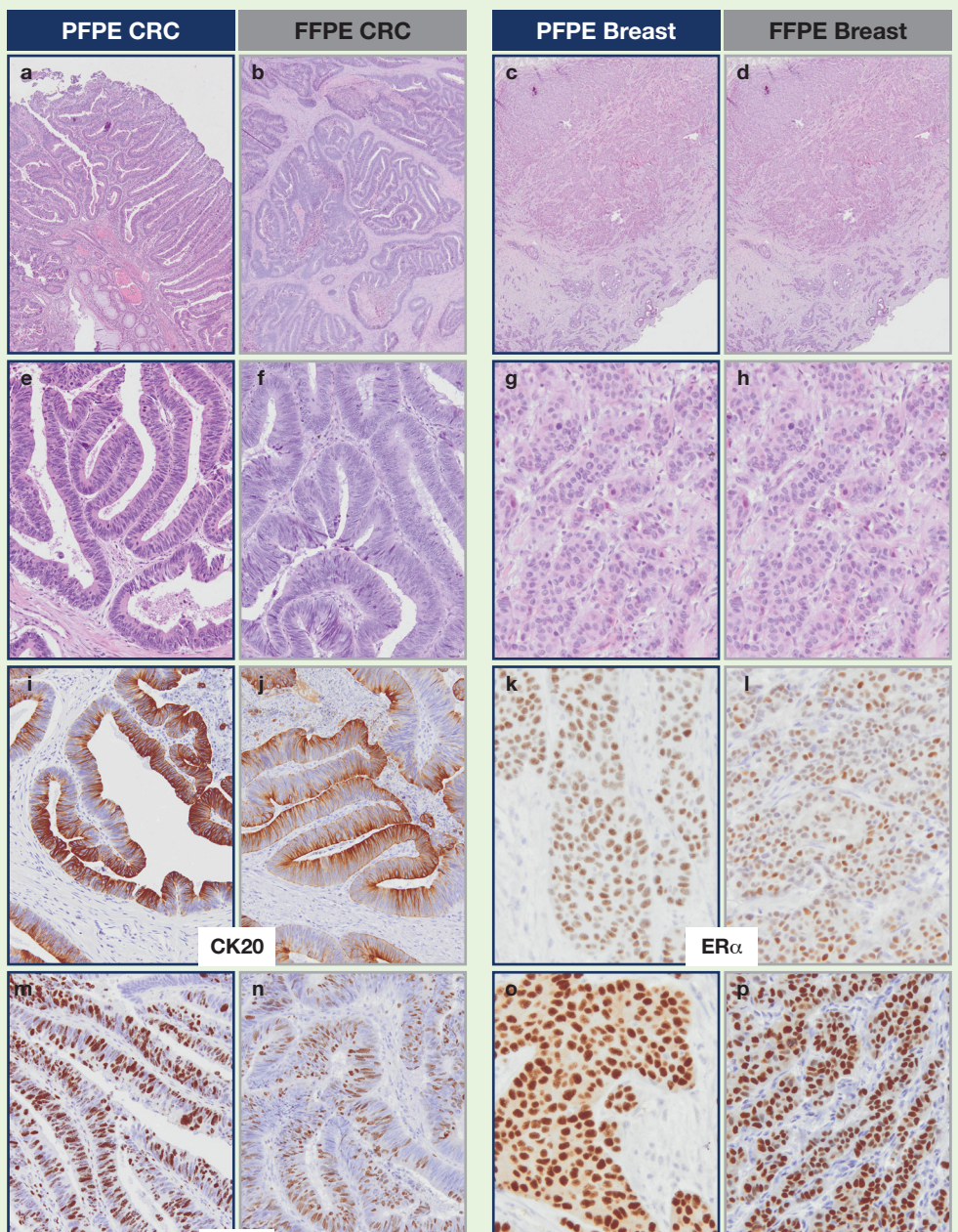

Figure 1. H&E and immunostaining of mirrored samples FFPE and PFPE from human cancer

Morphology of H&E stained human colorectal cancer (CRC) (a,b,e,f), and breast cancer (c,d,g,h); original magnifications x40 (a-d), x200 (e,f) and x400 (g,h).

Immunostaining of cytokeratin 20 (CK20) (i,j), Ki-67 (m,n), estrogen receptor alpha (ERα) (k,l) and progesteron receptor (PR) (o,p); original magnifications x200 (l,j,m,n), x400 (e,f) and x400 (k,l,o,p).

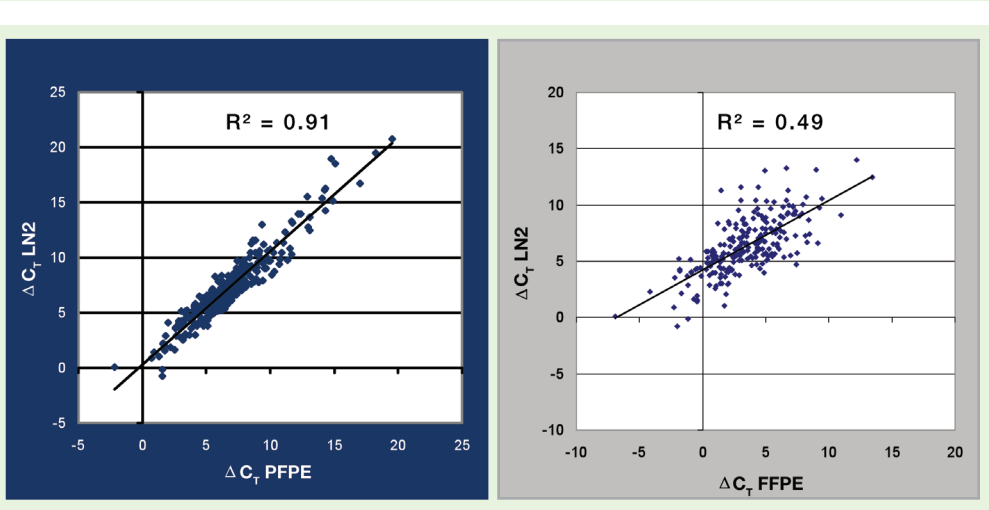

Figure 2. Gene expression analysis with RNA from mirrored samples of FFPE, PFPE and snap-frozen cancer specimens

Gene expression analysis by RT-qPCR with TaqMan Array Gene Signature 96-Well Plates (Life Technologies) with primer/probe assays of 92 genes associated with cancer or certain pathways and four housekeeping genes (HKG: 18s, GAPDH, HPRT1, GUSB). RNA (2 µg each) from mirrored samples of FFPE, PFPE and LN2 snap-frozen cancer specimens were used for cDNA synthesis. Arrays used: for breast cancer cases ´MAP kinases pathways plate, human´ and ´human molecular mechanism of cancer´ and for CRC cancer cases ´human colorectal cancer metastasis´.

C_T values were normalized with average C_T values from the four housekeeping genes: $\Delta C_T = C_T$ (target gene) – C_T (av. HKG); R^2: coefficient of determination.

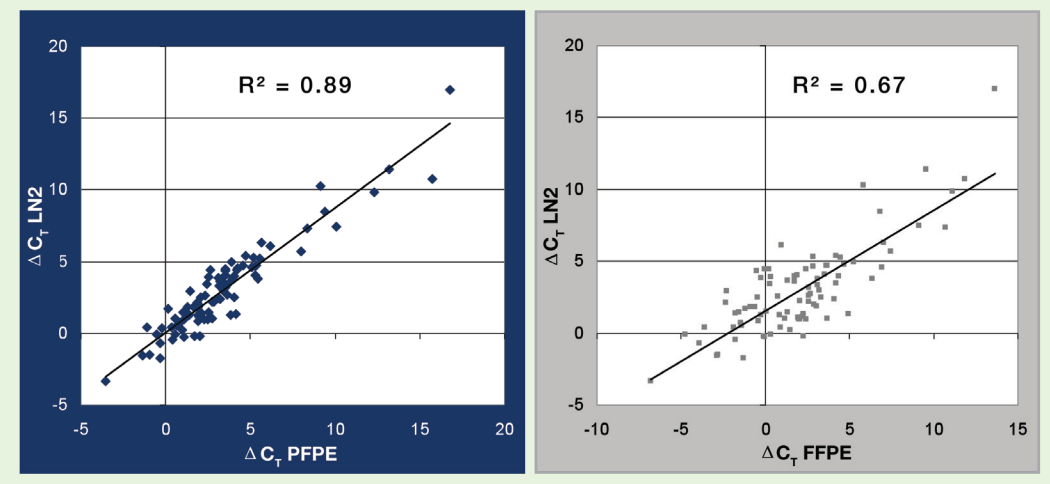

Figure 3. miRNA expression analysis of 88 different miRNA from mirrored samples of FFPE, PFPE and snap-frozen cancer specimens

miRNA expression analysis by RT-qPCR with RT² Profiler PCR Array Human miFinder from SABiosciences with SYBR-Green assays for 88 different miRNAs and for four house-keeping genes (HKG: SNORD 44, 47, 48 and U6). RNA (1 µg each) from mirrored samples of FFPE, PFPE and LN2 snap-frozen colorectal cancer specimens were used with RT² miRNA First Strand Kit followed by amplification with RT² Green/Rox qPCR Master Mix (SABiosciences).

C_T values were normalized with average C_T values from the four housekeeping genes: $\Delta C_T = C_T$ (target miRNA) – C_T (av. HKG); R^2: coefficient of determination.

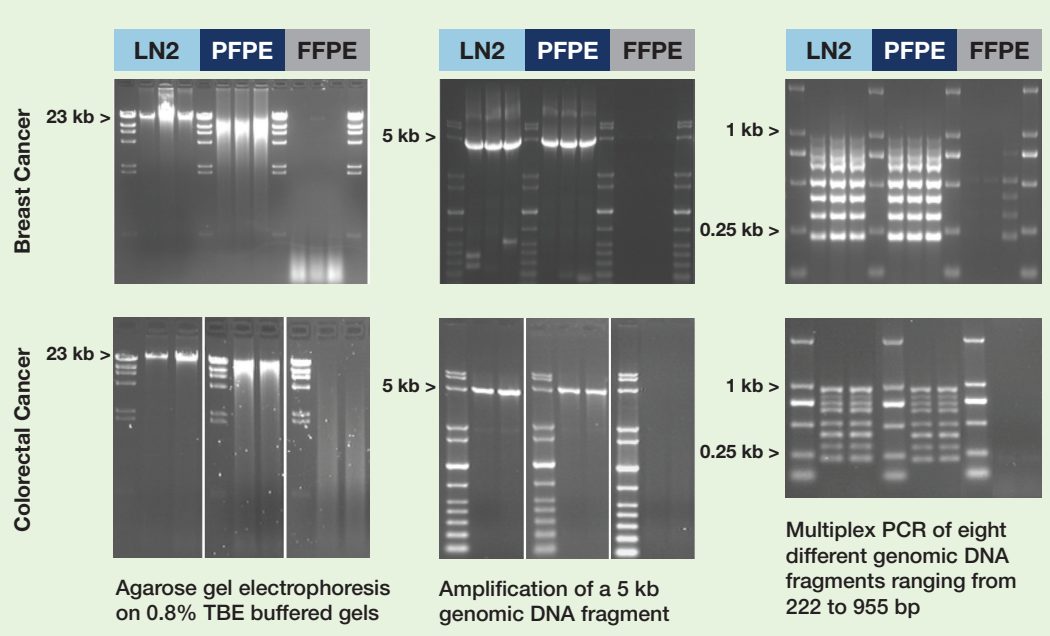

Figure 4. Integrity, long-range and multiplex PCR with DNA from mirrored samples of FFPE, PFPE and snap-frozen cancer specimens

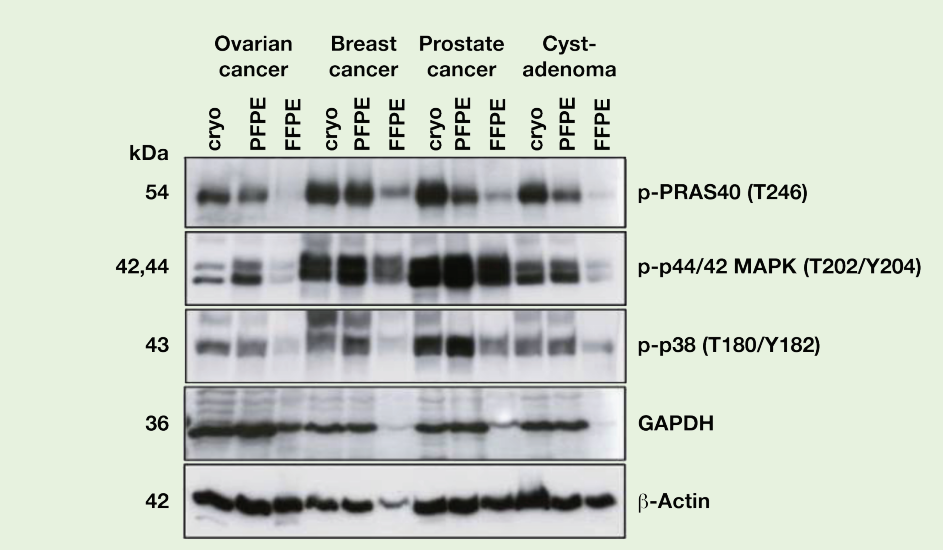

Figure 5. Preservation of the phosphoproteome in PFPE human tissue samples

Protein was extracted from mirrored samples of FFPE, PFPE and LN2 snap-frozen (cryo) malignant (ovarian, breast and prostate cancer) and non-malignant (cystadenoma) human specimens using the Qproteome® FFPE Tissue kit (QIAGEN). After protein extraction, 15 µg protein per lane was separated by SDS-PAGE and western blot analysis was performed using indicated antibodies.

Conclusion

Data presented in this poster demonstrate that in PAXgene fixed, paraffin embedded (PFPE) tissue

- Morphology is preserved similarly to neutral buffered formalin.
- Sections of PFPE can be used for immunohistochemical staining.
- RNA and miRNA profiles are in high concordance with the profiles found in mirrored snap-frozen samples, while those of FFPE tissue correlate poorly to miRNA and RNA profiles in snap-frozen tissue controls.
- DNA of high molecular weight and full length, immunoreactive proteins, including phosphoproteins can be isolated from PFPE tissue.

References

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Conflict of interest statement:

Daniel Grölz, Nadine Dettmann, Isabell Blassnig and Ralf Wyrich are employed by QIAGEN. Lynne Rainen is employed by BD. All other authors have no conflict of interest.

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