Paxgene® Tissue: A New Tissue Fixation Technology for Simultaneous Preservation of Morphology and Nucleic Acids

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

[1]Daniel Grölz, [2]Nadine Dettmann, [2]Isabell Blassnig, [1]Ralf Wyrich and [1]Lynne Rainen

[1]PreAnalytiX GmbH, Hombrechtikon, Switzerland; [2]QIAGEN GmbH, Hilden, Germany

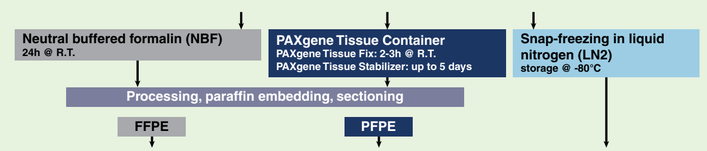
Introduction

PAXgene Tissue is a new, formalin-free fixation technology developed for simultaneous preservation of morphology and biomolecules in tissue samples[1].

In this study, cases of human lung, breast, and colorectal cancer were divided and fixed in formalin, PAXgene Tissue, or snap frozen in liquid nitrogen (LN2). Formalin fixed, paraffin-embedded (FFPE) and PAXgene fixed, paraffin-embedded (PFPE) tissue morphologies were compared using H&E stain. RNA, miRNA and DNA were isolated from FFPE, PFPE, and LN2 tissue. RNA expression was analyzed in 96-well RT-qPCR arrays with pre-designed assays; miRNA expression profiling was performed with single Sybr-green or primer/probe based assays. DNA was analyzed by agarose gel electrophoresis, long-range PCR, and multiplex PCR.

Cancer Cases & NA Purification Methods

Breast cancer case	Infiltrative ductal carcinoma, grade G2, stage T2N2M0, 4/17 lymph nodes positive
Lung cancer case	Squamous cell lung carcinoma, grade G2, stage T3 N0 M0
Colon cancer	Colorectal cancer



RNA	RecoverAll™ Total Nucleic Acid Isolation Kit – Optimized for FFPE Samples (Ambion)	PAXgene Tissue RNA	RNeasy® Mini
miRNA		PAXgene Tissue miRNA	miRNeasy® Mini
DNA	QIAamp® DNA FFPE	PAXgene Tissue DNA	QIAamp DNA Mini

Results

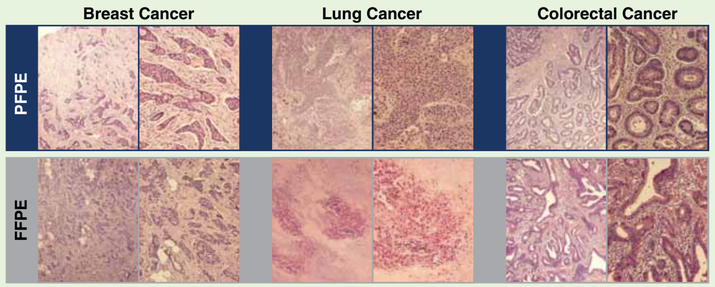


Figure 1. Hematoxylin and Eosin (H&E) Stained Sections

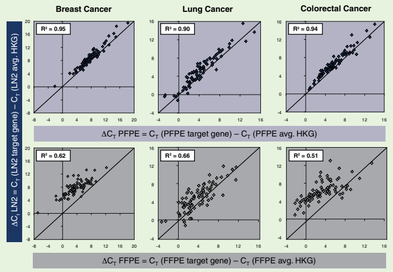


Figure 2. mRNA Expression Analysis

Scatterplots with ΔC_T values from PCR arrays: ΔC_T FFPE or ΔC_T PFPE *versus* ΔC_T from snap-frozen (LN2) tissue; RT-qPCR with RNA from mirrored samples of FFPE, PFPE and LN2 snap-frozen cancer specimens. C_T values were normalized with average C_T values from housekeeping genes (HKG): $\Delta C_T = C_T$(target gene) – C_T(avg. HKG); R^2: coefficient of determination.

Arrays used:

Breast cancer case: TaqMan® Array Gene Signature 96-Well Plate ´MAP kinases pathways plate, human´ (Applied Biosystems) with primer/probe assays of 92 genes associated with cancer and 4 HKG. 2 µg RNA used for cDNA synthesis.

Lung cancer case: RT² Profiler™ PCR Array ´human epithelial to mesenchymal transition´ (SABiosciences) with Sybr-Green assays of 84 genes involved in this process and 5 HKG; 100 ng RNA from FFPE and PFPE with 8 cycle pre-amplification (RT² FFPE PreAMP cDNA synthesis and Primer Mix from SABiosciences); 300 ng of RNA from LN2 without preamplification.

CRC case: TaqMan Array Gene Signature 96-Well Plate ´human colorectal cancer metastasis´ (Applied Biosystems) with primer/probe assays of 92 genes associated with cancer and 4 HKG. 2 µg RNA used for cDNA synthesis.

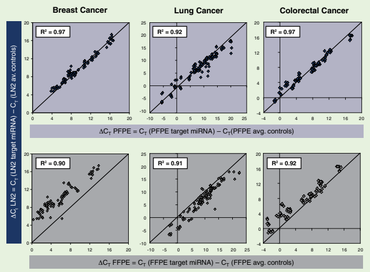


Figure 3. miRNA Expression Analysis

Scatterplots with ΔC_T values from 15 different single miRNA specific RT-qPCR assays: ΔC_T PFPE or ΔC_T FFPE *versus* ΔC_T from LN2; C_T values were normalized with average C_T values from six control genes: $\Delta C_T = C_T$(target gene) – C_T(avg. controls); R^2: coefficient of determination.

5ng RNA from triplicate purifications were used for each primer/probe TaqMan microRNA assay for miR10a, -16, -29a, -30b, -103, -192 (Applied Biosystems).

200ng RNA from triplicate purifications were used for cDNA synthesis and amplification in Sybr green miScript assays miR9, -10a, -10b, -29a, -103, -125b, -143, -145, -192 and miScript PCR controls RNUA1, RNU5A, RNU6B, SNORD25, SCARNA17, SNORA73A (QIAGEN).

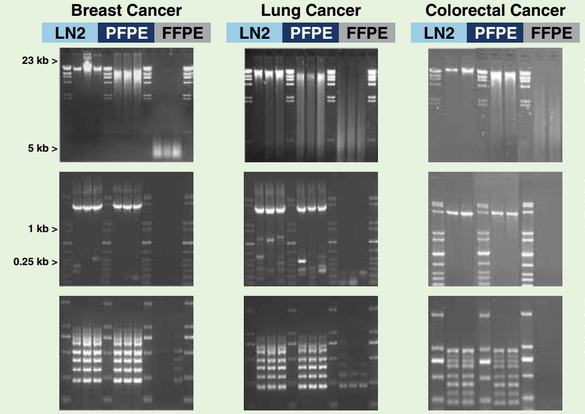


Figure 4. Long-range and Multiplex PCR. DNA isolated from LN2, PFPE, and FFPE tissues.

Conclusion

- Morphology of PFPE tissue was similar to or indistinguishable from FFPE tissue.
- A high correlation of RNA gene expression results was observed between RNA from PFPE and LN2 fixed tissue. In contrast, poor correlation was observed between RNA from FFPE and LN2 fixed tissue.
- Correlation of miRNA expression in FFPE and LN2 fixed tissue was high but not as high as between PFPE and LN2 fixed tissue.
- High molecular weight DNA which performed well in PCR could be isolated from PFPE and LN2 fixed tissue. DNA from FFPE tissue was of lower molecular weight, failed in long-range PCR and showed biased or no amplification in multiplexed PCR.

Summary

The PAXgene Tissue fixation technology preserves both morphology and nucleic acids in tissue samples. It enables morphological and multimodal biomarker testing with one sample.

References

1. Bilge, E.; Meding, S.; Langer, R.; Kap, M.; Viertler, C.; Schott, C.; Ferch, U.; Riegman, P., Zatloukal, K.; Walch, A.; Becker K-F. Proteomic Analysis of PAXgene-Fixed Tissues. *J Proteome Res.* **2010** Oct 1; 9(10):5188-5196

Acknowledgments

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