Preservation of Gene Expression Profile and Histomorphology in Human Breast Tumor Tissue with the New PAXgene® Tissue System

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Introduction

PreAnalytiX has developed a system for preservation of histomorphology and nucleic acids in paraffin embedded tissue samples. The system is comprised of a collection container for formalin-free fixation and stabilization of tissue specimens and specialized purification kits for isolation of DNA, RNA, or microRNA (miRNA) from PAXgene Tissue fixed, paraffin embedded (PFPE) tissue samples.

In this case study, a tissue specimen of human infiltrating ductal carcinoma (IDC) of the breast was divided into three parts after resection and 1) fixed in neutral buffered formalin (NBF), 2) fixed and stabilized in the PAXgene Tissue Container, or 3) snap frozen in liquid nitrogen (LN2). Paraffin embedded tumor samples were compared for preservation of histomorphology, expression of ERα, PR, and HER2. RNA was isolated from paraffin embedded and snap frozen samples and compared for integrity and preservation of the gene expression profile.

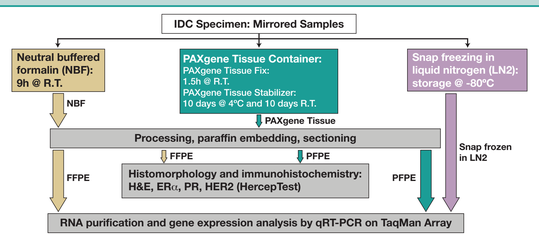
Materials and Methods

Tissue specimen	Histologic type: Infiltrating ductal carcinoma of the breast Histologic grade: moderately differentiated

NBF	4% neutral buffered formalin
PAXgene Tissue	PAXgene Tissue Container
RNA Isolation, FFPE	PureLink™ FFPE Total RNA Isolation Kit (Invitrogen)
RNA Isolation, PFPE	PAXgene Tissue RNA Kit (PreAnalytiX)
*IHC for HER2 antigen	HercepTest™ (Dako)
*IHC for ERα	Anti-Human Estrogen Receptor a, clone 1D5 (Dako)
*IHC for PR	Anti-Human Progesteron Receptor, clone 1A6 (BIOPRIME)
qRT-PCR	TaqMan® Array Gene Signature 96-Well Plate: human molecular mechanism of cancer (Applied Biosystems)

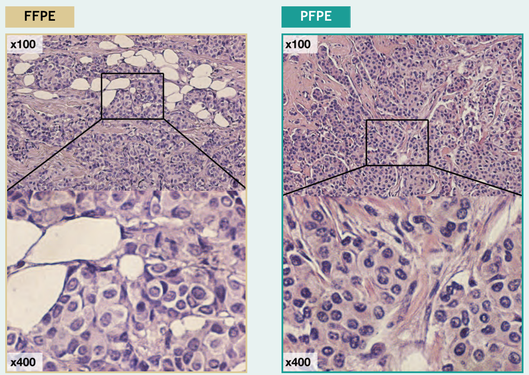
*Immunohistochemical assay

Study Design



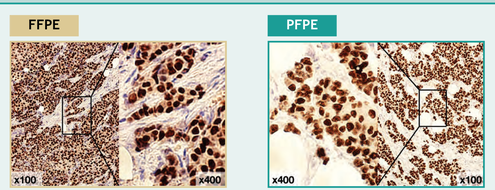
Results

Figure 1: H&E staining



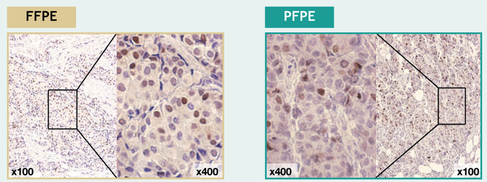
Hematoxylin and eosin (H&E) stained sections. Mirrored samples of FFPE and PFPE human breast cancer.

Figure 2: IHC analysis of ERα (Estrogen Receptor alpha) expression



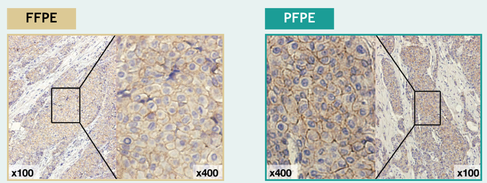
Uniform positive reaction for estrogen receptor in IHC staining of ERα antigen in a labeled streptavidin-biotin assay counterstained with hematoxylin. Mirrored samples of FFPE and PFPE human breast cancer.

Figure 3: IHC analysis of PR (Progesterone Receptor) expression



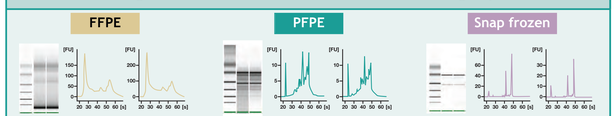
Heterogeneous staining reaction for progesterone receptor in IHC staining of PR antigen in a labeled streptavidin-biotin assay counterstained with hematoxylin. Mirrored samples of FFPE and PFPE human breast cancer.

Figure 4: IHC analysis of HER2



IHC staining of HER2 with HercepTest (Dako). Assay performed according to manufacturer's instructions. Mirrored samples of FFPE and PFPE human breast cancer. For the PFPE sample, antigen retrieval was not needed and was omitted.

Figure 5: RNA Isolation - Yield and Integrity



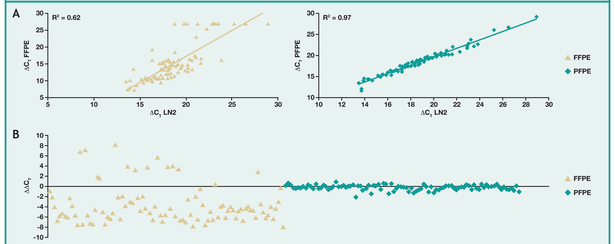
RIN	2.2	2.1
Yield/ 3x 10 µm	1.9 µg	2.9 µg

RIN	5.8	5.4
Yield/ 3x 10 µm	4.6 µg	4.5 µg

RIN	9.9	9.4
Yield/ 10 mg	6.2 µg	3.1 µg

RNA isolation in duplicate from mirrored samples of FFPE, PFPE, and LN2 snap frozen human breast cancer specimen. Digital gel, electropherogram, and RNA integrity number (RIN) obtained with Agilent 2100 Bioanalyzer. Yield measured by spectrophotometric analysis with a NanoDrop® instrument.

Figure 6: Gene expression analysis on TaqMan Array Gene Signature 96-Well Plate 'human molecular mechanism of cancer'



Quantitative real time RT-PCR with primer/probe assays of 92 genes associated with cancer and 4 housekeeping genes, including 18S rRNA.

$\Delta^*C_T = C_T$(target gene) - C_T(18S rRNA); $\Delta\Delta C_T = \Delta C_T$ (FFPE or PFPE) - ΔC_T (snap frozen). A: Scatterplot of ΔC_T values for 95 genes: ΔC_Ts FFPE or ΔC_Ts PFPE vs ΔC_Ts from snap-frozen tissue, R^2: coefficient of determination; B: $\Delta\Delta C_T$s for 95 genes, each with RNA from FFPE and PFPE specimen; all RNAs were isolated from mirrored samples of FFPE, PFPE, and LN2 snap-frozen human breast cancer specimens. *C_T: cycle threshold

Conclusions

In conclusion, the test results yielded the following comparison between PFPE and FFPE breast tumor (IDC) tissue:

- H&E stained sections of PFPE were similar to, or indistinguishable from, FFPE tissue.
- Immunohistochemical staining of HER2, ERα, and PR gave comparable staining intensities in PFPE and FFPE samples.
- RNA from LN2 snap frozen and PFPE samples were of high integrity with average RIN values of 9.5 and 5.6 respectively, compared to low RIN value of 2.2 from FFPE.
- Gene expression analysis of 96 genes in real time RT PCR showed a high correlation of C_T (cycle threshold) values for the samples from LN2 snap frozen and PFPE tissue (R^2=0.97), and a poor correlation between the samples from LN2 snap frozen and FFPE (R^2=0.62) tissue.

Summary

The PAXgene Tissue System preserves morphology and gene expression profile in paraffin embedded breast cancer tissue samples. Histomorphology is preserved similarly to that seen in FFPE tissue, while the gene expression profile shows a high correlation to snap frozen tissue.