

Comparison of Methods for Preserving Morphological, Molecular, and Protein Biomarkers in Tissue

Lynne Rainen, Ph.D.
Scientific Director
PreAnalytiX GmbH

Sample Prep and Target Enrichment in Molecular Diagnostics
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Presentation Outline

- Current tissue preservation methods
- Introduction to the PAXgene® Tissue System
- Comparison of PAXgene Tissue to FFPE and fresh frozen tissue
 - Morphology
 - RNA, DNA, miRNA
 - Proteins
- Acknowledgements
- Questions

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Current methods do not simultaneously preserve tissue morphology and biomolecules.

Neutral buffered formalin (NBF)

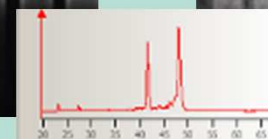
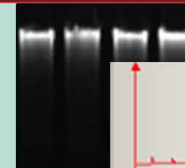
Liquid nitrogen or RNAlater™

Morphology Preservation:



Two samples needed for high quality biomolecules and histomorphology.

Nucleic Acid Preservation:

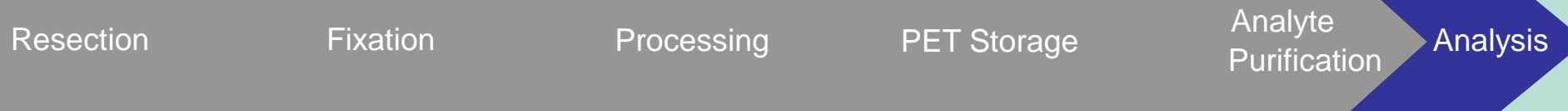


Drawbacks:

- Crosslinking of biomolecules
- Lack of standardization

- Morphology destroyed or compromised

Preanalytical variables highly influence sample quality.



- Ischemia time
 - Fixation conditions
 - Transport conditions
 - Processing/embedding temperatures
 - PET storage time/temperature
 - Biomolecule isolation protocol

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The *PAXgene Tissue System consists of a specimen container and companion nucleic acid isolation kits.

PAXgene Tissue Container



PAXgene Tissue Kits



RNA & miRNA



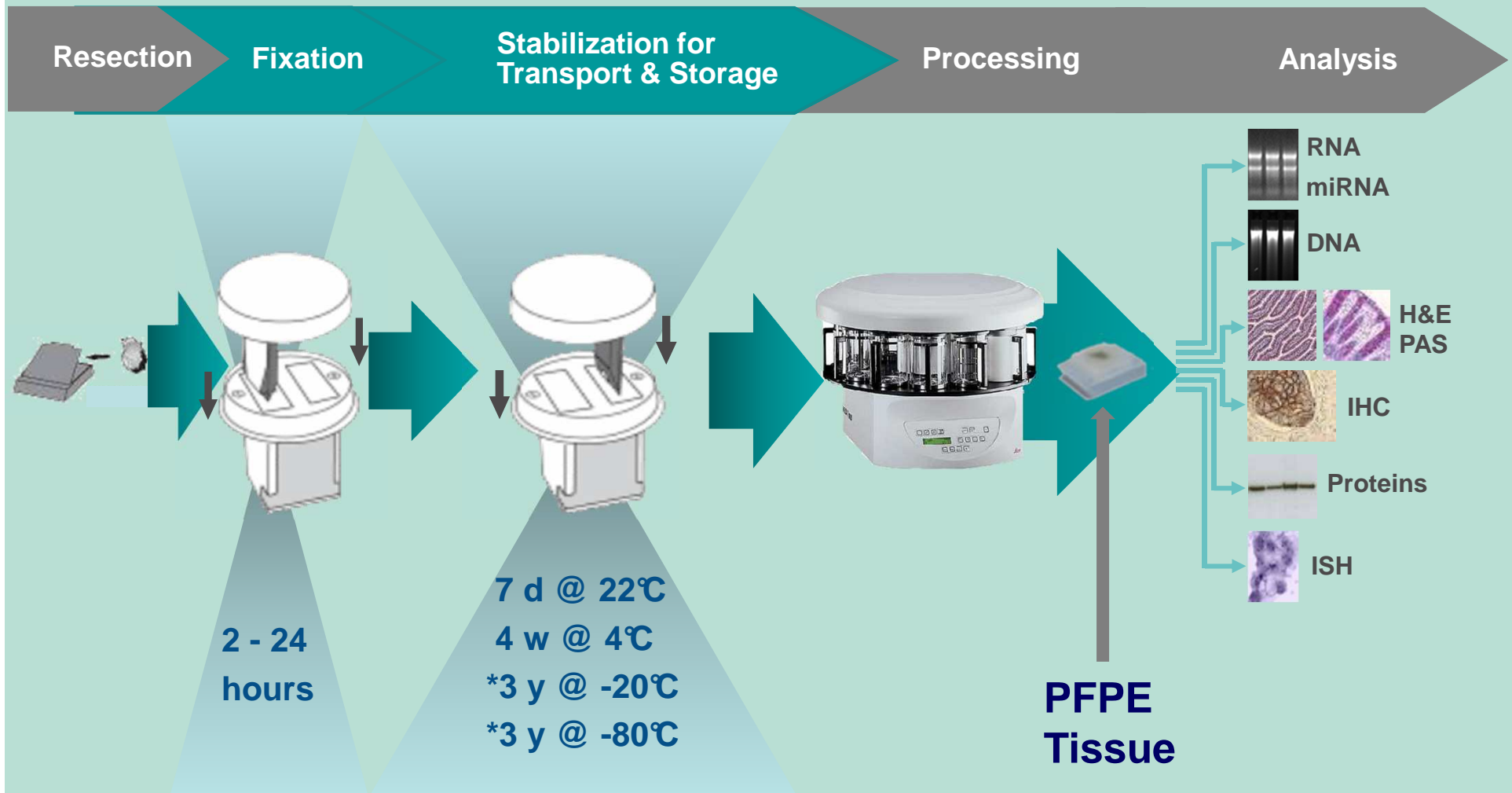
RNA



DNA

*For research use only. Not for use in diagnostic procedures.

The PAXgene Tissue System simultaneously preserves tissue morphology, DNA, RNA, miRNA and proteins.

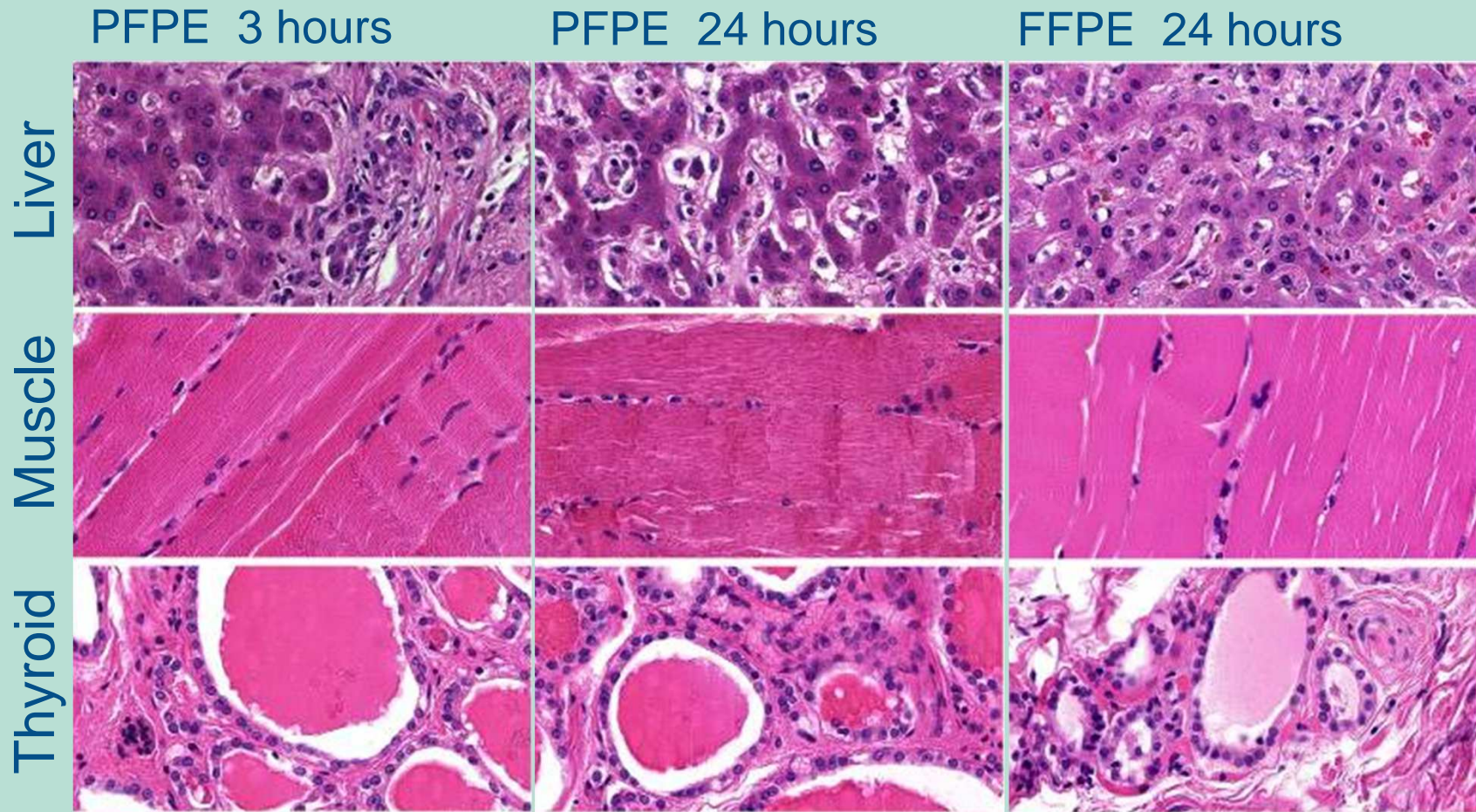


*Studies ongoing.

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Tissue can be fixed in as little as two hours or up to 24 hours in PAXgene with no adverse effect on morphology.

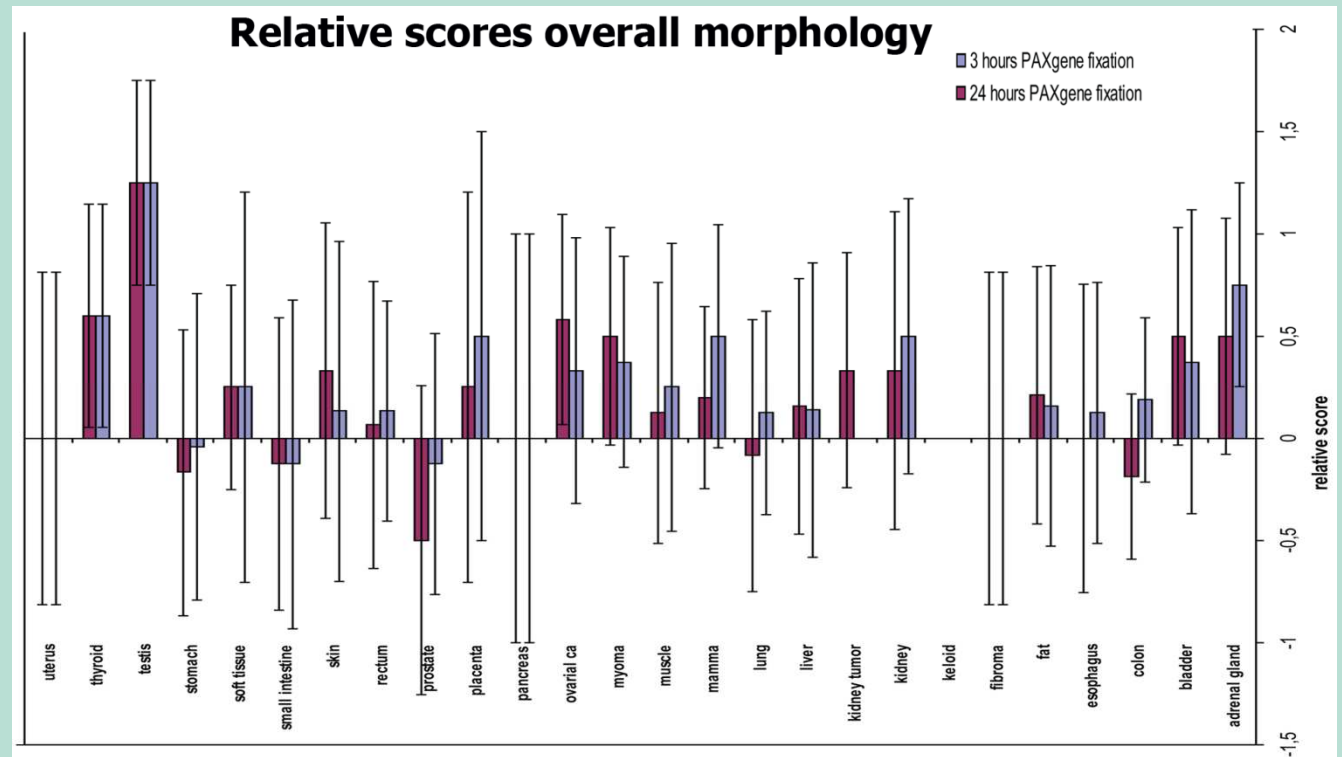


<http://www.preanalytix.com/product-catalog/tissue/tissue-atlas/>

Pathologists grade PAXgene fixed tissue equal to or better than formalin-fixed tissue for morphology.

“Histological Assessment of PAXgene Tissue Fixation and Stabilization Reagents”

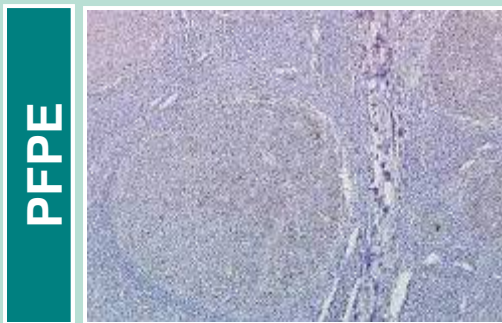
- 26 human tissue types
- Mirrored samples: FFPE and PFPE
- Evaluation performed by four pathologists from three clinical institutions
- Scoring system: **-2 to +2 relative to FFPE**
 - ✓ Nuclear details
 - ✓ Cytoplasmic details
 - ✓ Membrane details
 - ✓ Contrast
 - ✓ Overall impression



IHC can be optimized in PAXgene fixed tissue to yield results comparable to formalin-fixed tissue.

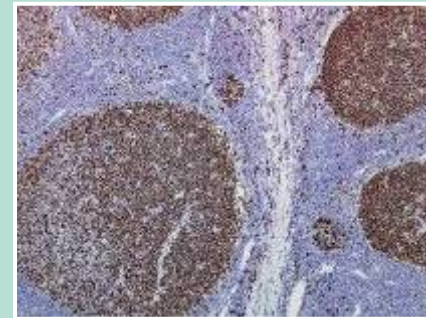
IHC of Human tonsil:

Ki-67, clone MIB-1, labeled streptavidin-biotin (LSAB) assay

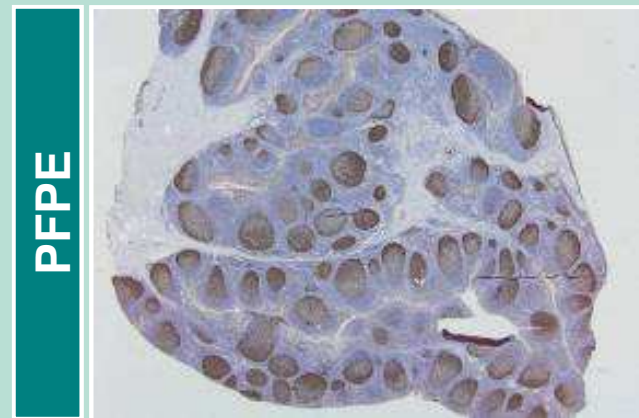
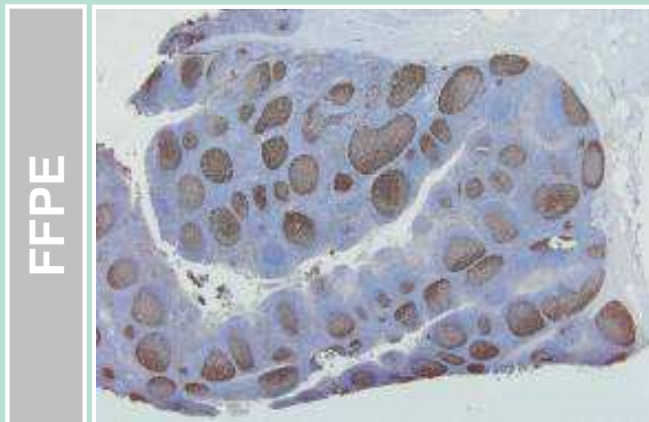


20 min 98°C,
citrate buffer pH 6

Optimization
→



10 min 70°C,
Tris/EDTA buffer pH 9



PFPE tissue is comparable to FFPE tissue in ER α and PR IHC assays.

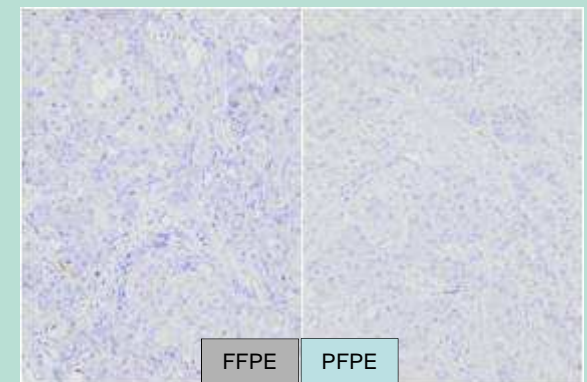
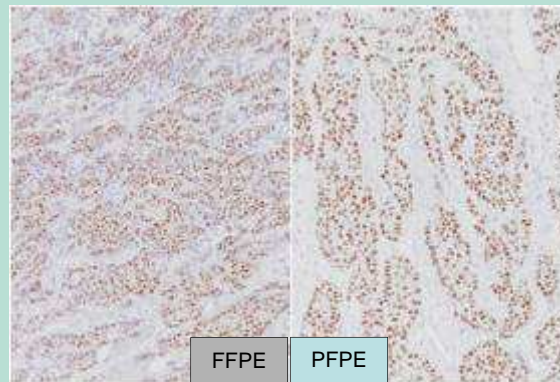
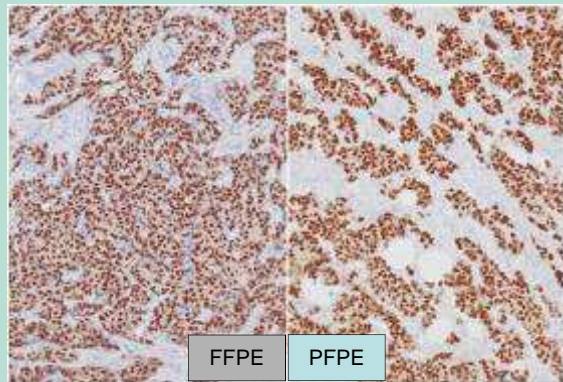
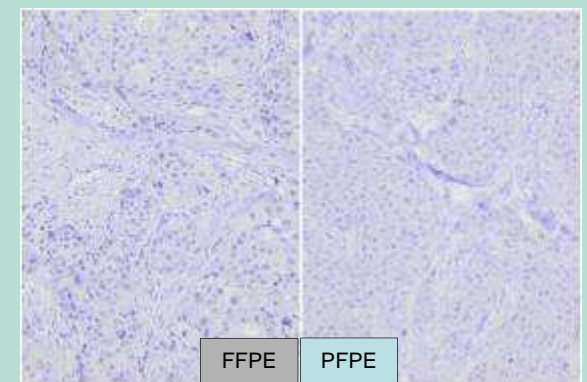
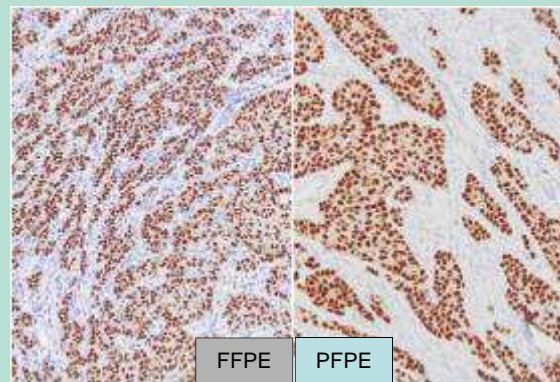
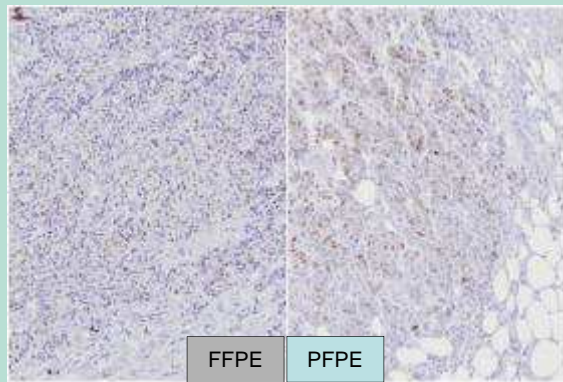
IHC of Human Breast cancer: labeled streptavidin-biotin (LSAB) assay

Case 1

Case 2

Case 3

ER α
(1D5)

PR
(1A6)

www.preanalytix.com/.../tissue-atlas

PFPE tissue is comparable to FFPE tissue in HER2 SPOt-Light® CISH assay.

Hu Br Ca:

Cases 1 and 2: Her2 +++

Cases 3 and 4: Her2 +

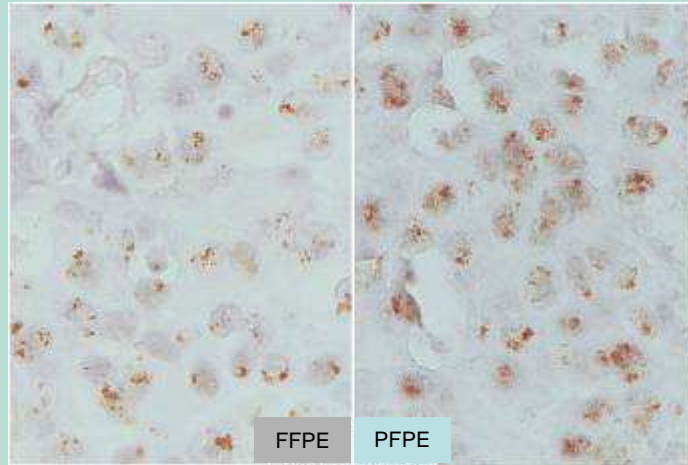
CISH Conditions FFPE:

- 15 min 98°C
- 5 min enzymatic digestion

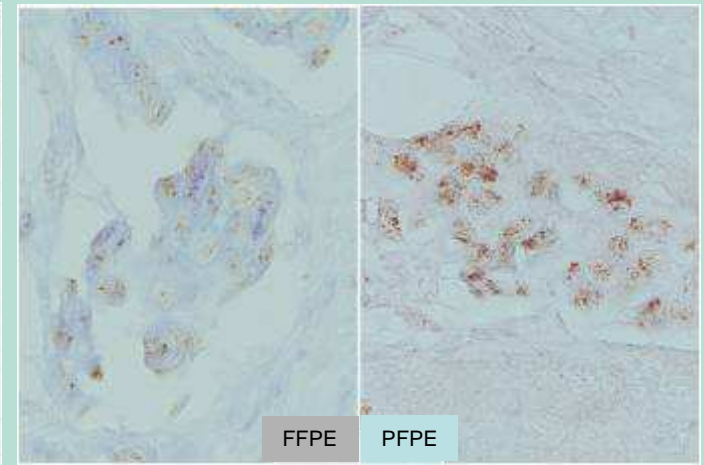
CISH Conditions PFPE:

- no heat pretreatment
- no enzymatic digestion

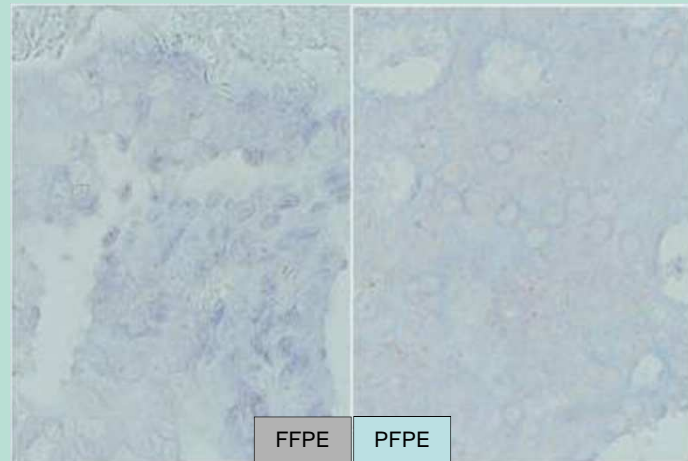
Case 1



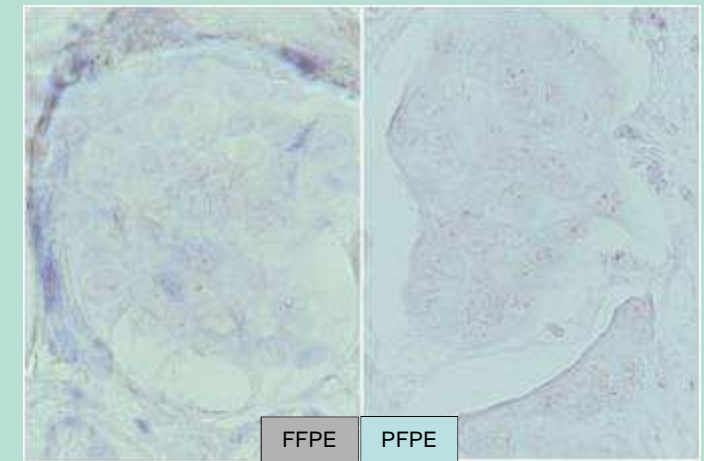
Case 2



Case 3



Case 4



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The PAXgene Tissue System maintains tissue morphology while preserving RNA integrity.

4 hours fixation, 7 days stabilization at 22°C

Liver

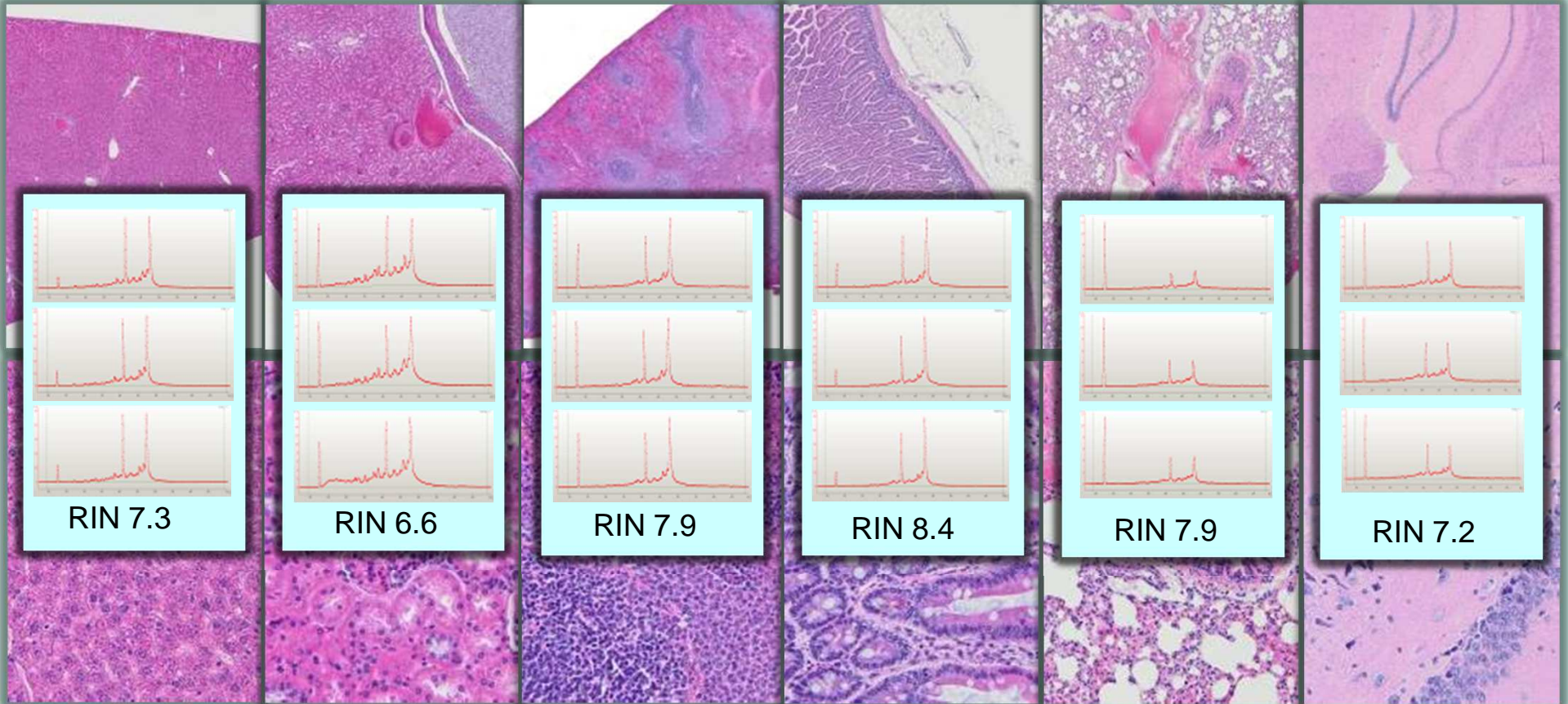
Kidney

Spleen

Intestine

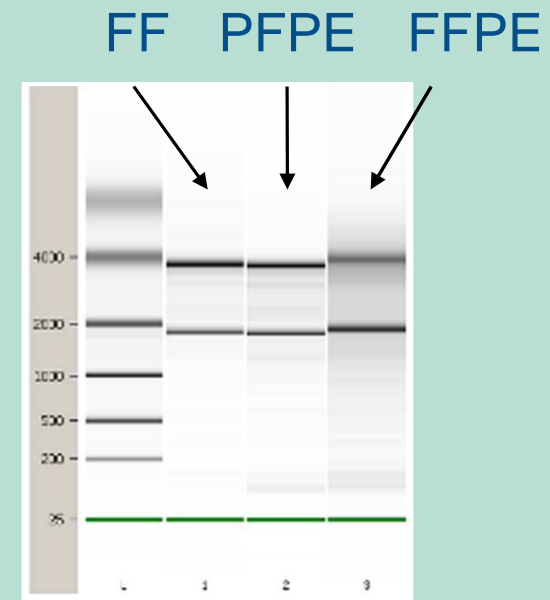
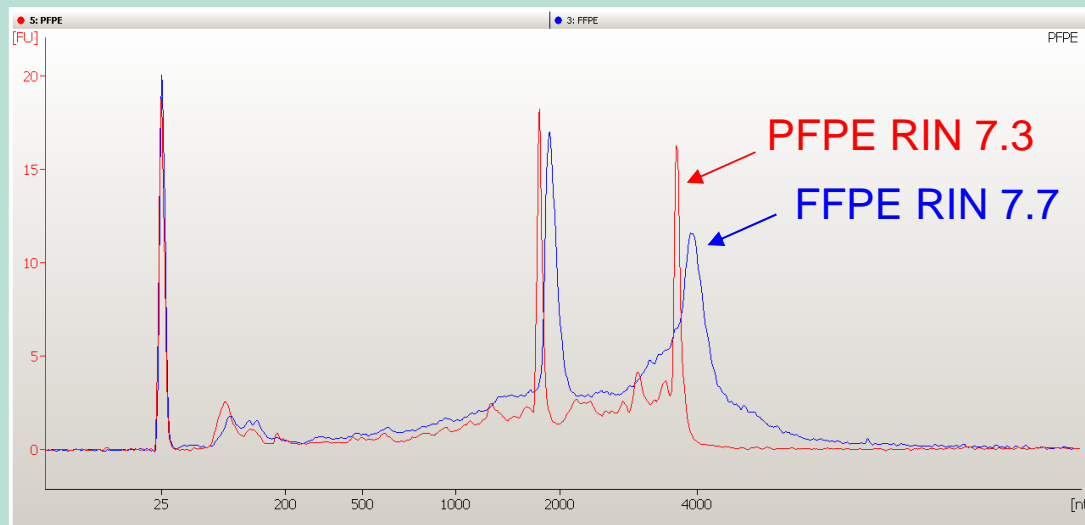
Lung

Brain



RNA from the PAXgene Tissue System is free of chemical modifications.

Agilent Bioanalyzer Results of RNA from Rat Liver



FF = fresh frozen

PFPE = PAXgene fixed, paraffin-embedded

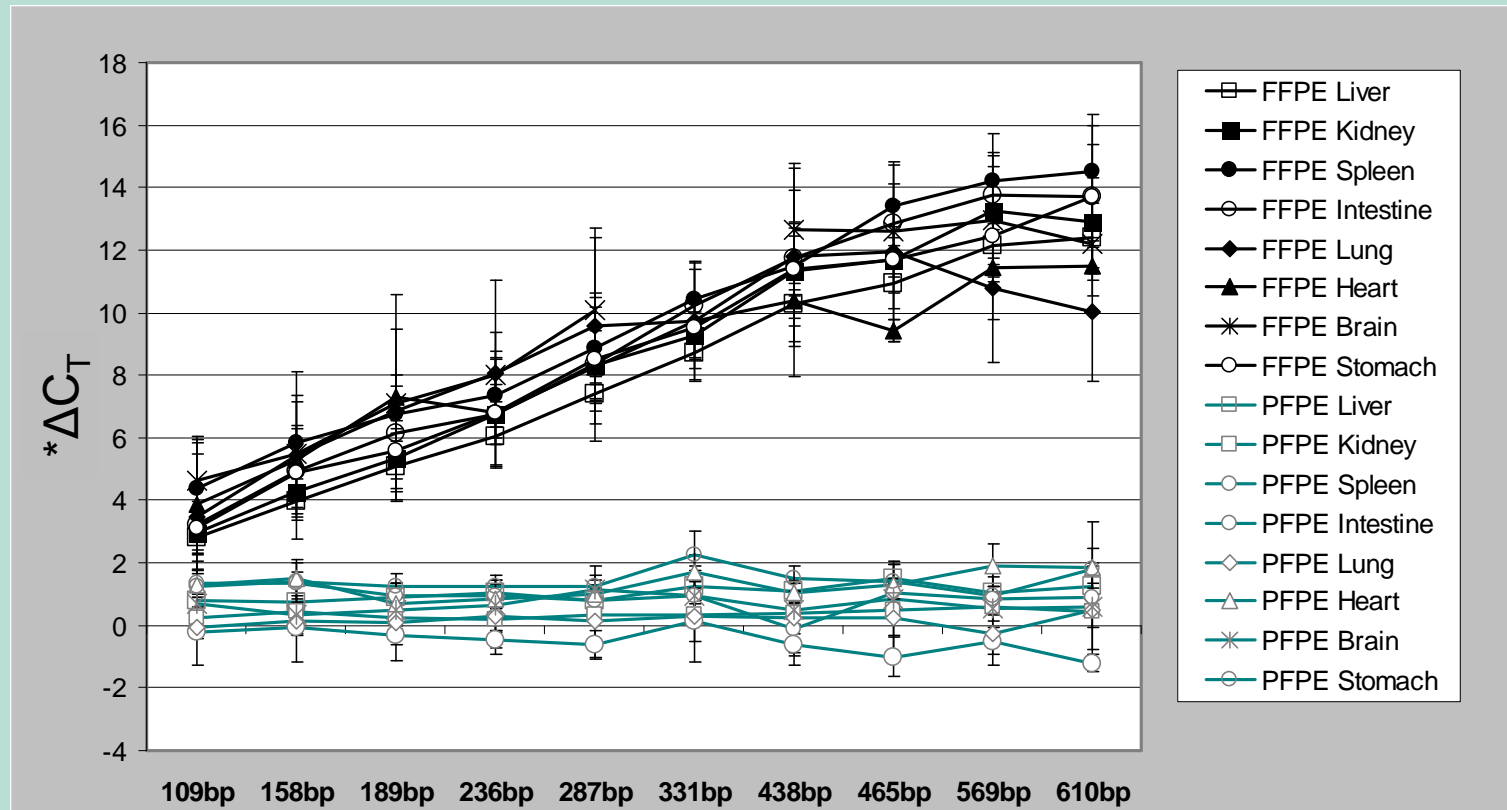
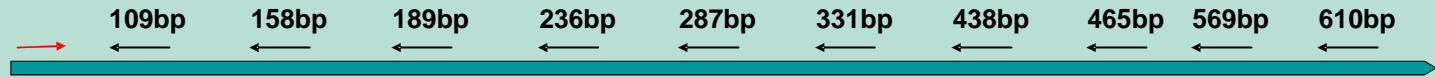
FFPE = formalin-fixed, paraffin-embedded

Groelz, D., Sobin, L., Branton, P., Compton, C., Wyrich, R., Rainen, L. 2013 Experimental and Molecular Pathology , 94 (1) pp. 188 - 194 .

- 17 -

RNA from the PAXgene Tissue System performs better in RT-PCR than RNA from formalin-fixed tissue.

Primers β -Actin gene



* $\Delta C_T = C_T(\text{RNA from PFPE or FFPE}) - C_T(\text{RNA from FF})$.

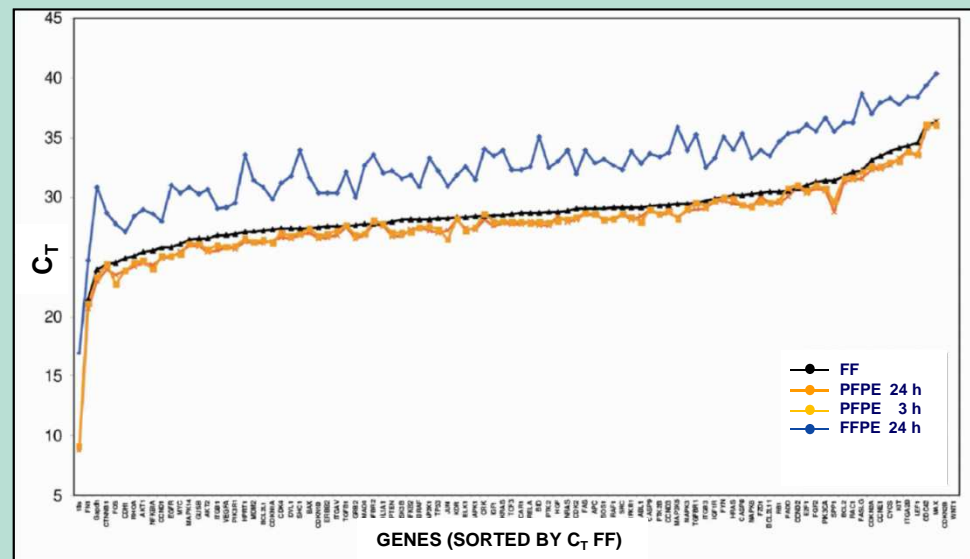
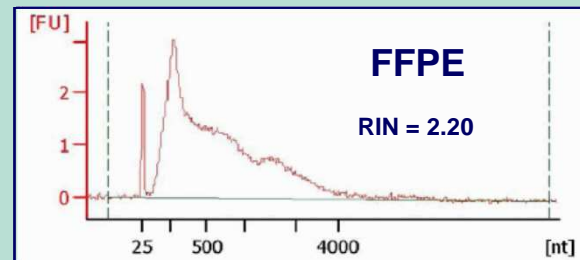
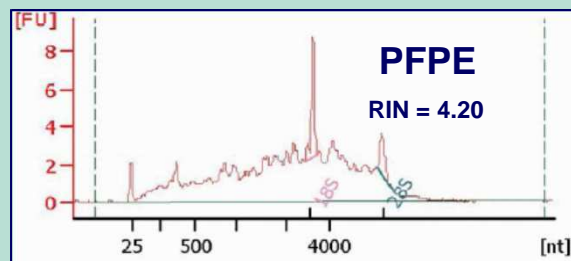
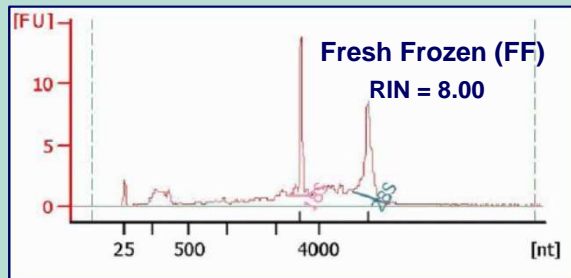
Groelz, D., Sobin, L., Branton, P., Compton, C., Wyrich, R., Rainen, L. 2013 Experimental and Molecular Pathology , 94 (1) pp. 188 - 194 .

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PAXgene fixed tissue RNA compares to RNA from fresh frozen tissue in multiplexed RT-qPCR assays.

“A New Technology for Stabilization of Biomolecules in Tissues for Combined Histological and Molecular Analyses”

RNA from human liver



Summary of > 800 RT-qPCR assays

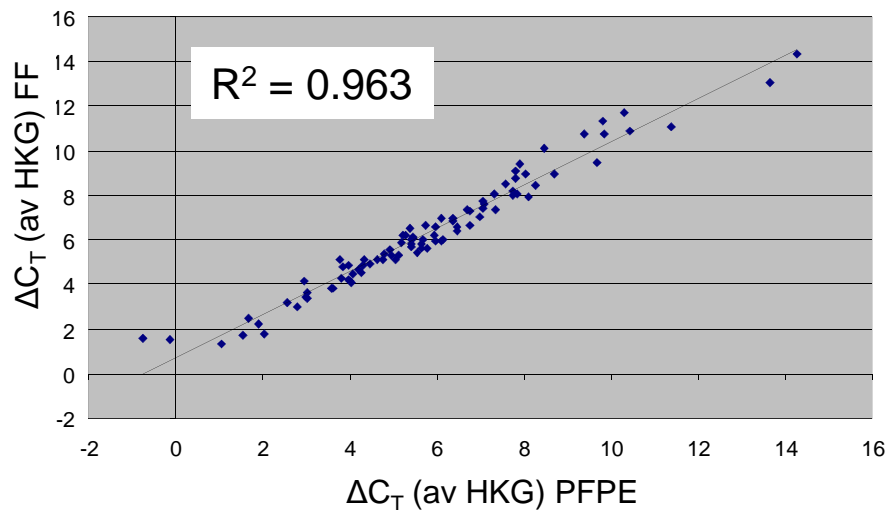
Viertler C, et al., J Mol Diagn. 2012 Sep;14(5):458-66.

- 19 -

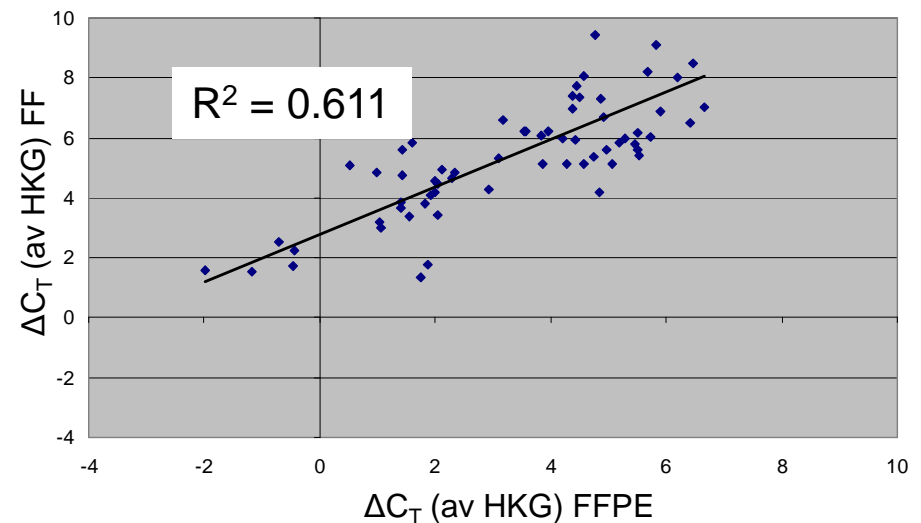
PFPE tissue is comparable to FF tissue in gene expression *array for molecular mechanisms of cancer.

Combined gene expression array results for three human breast cancer samples.

PFPE vs. FF



FFPE vs. FF



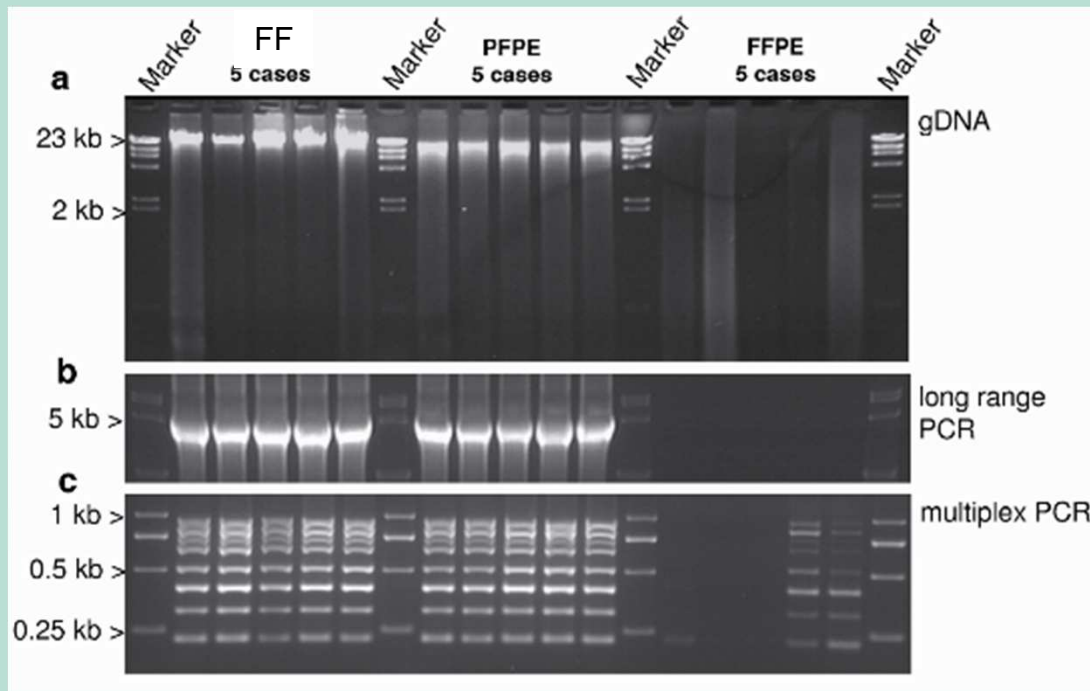
*LifeTechnologies Human Molecular Mechanisms of Cancer Gene Expression Array

The quality and performance of DNA from PAXgene fixed tissue compares to DNA from fresh frozen tissue.

Human ileum



Human colorectal cancer (CRC)

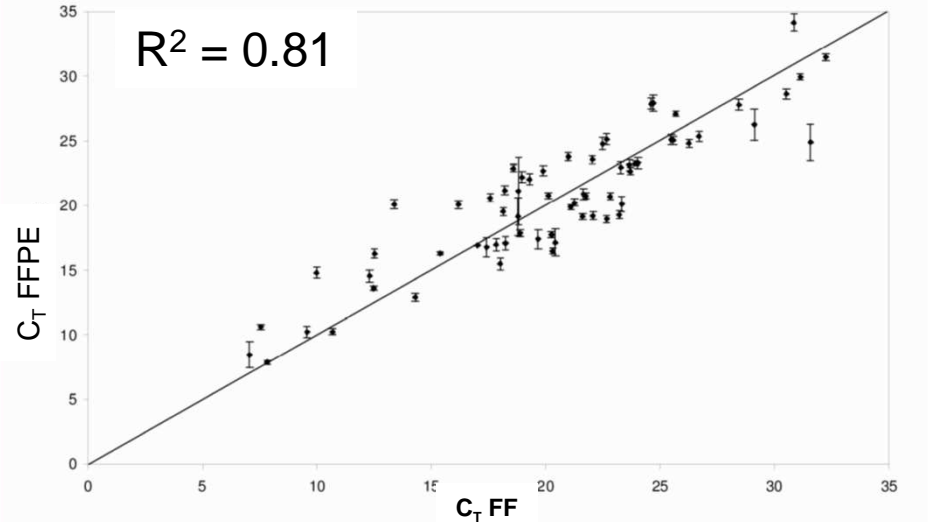
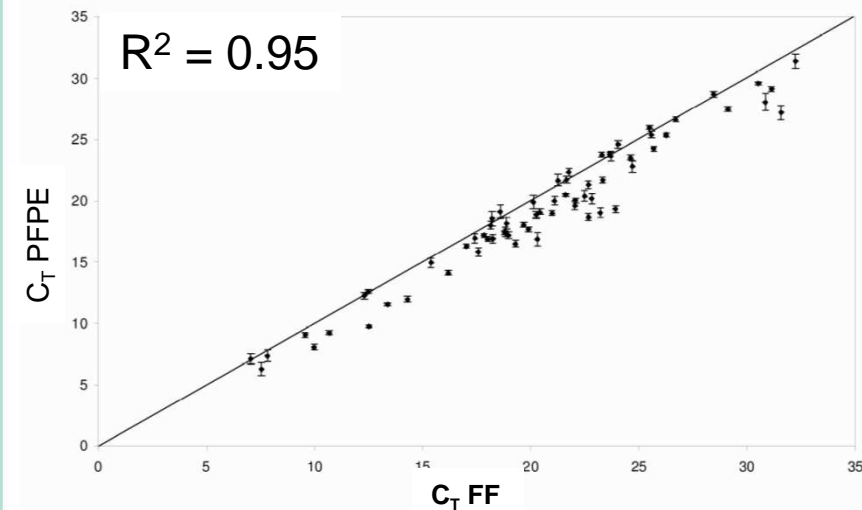


Viertler C, et al., J Mol Diagn. 2012 Sep;14(5):458-66.

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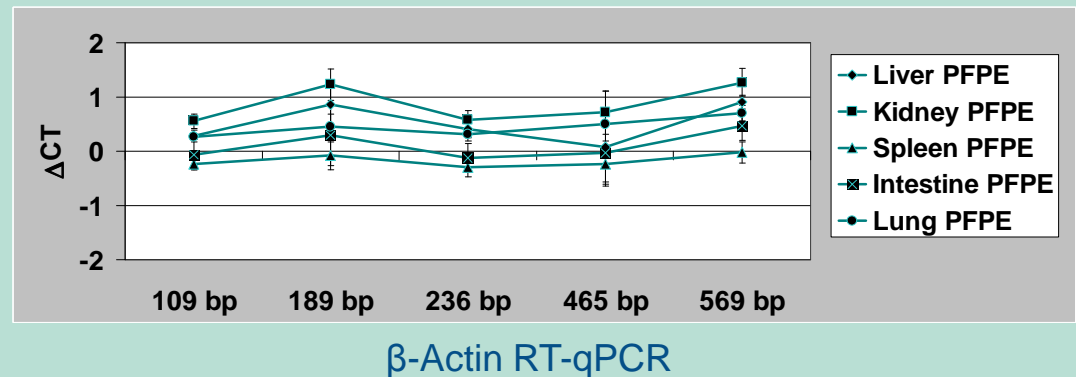
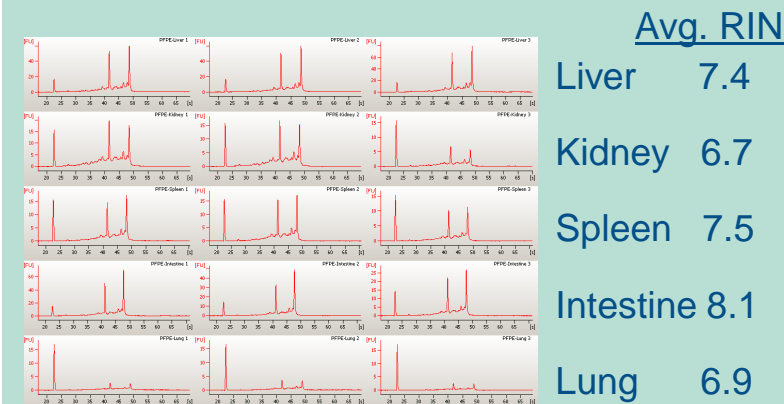
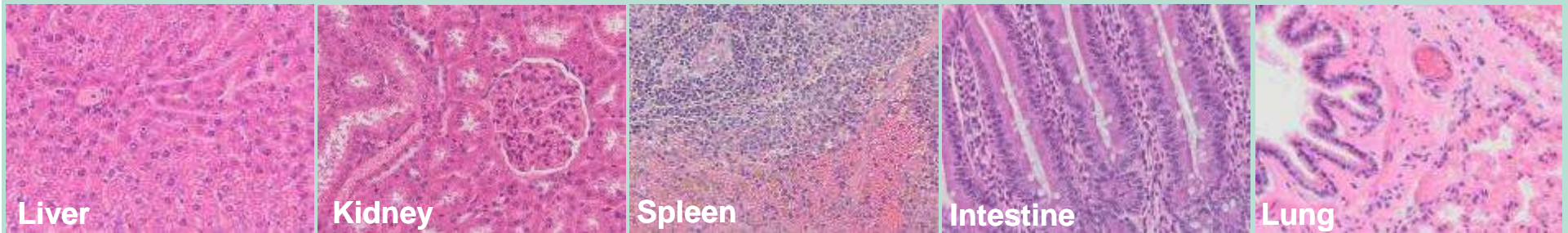
miRNA from PAXgene fixed tissue is comparable to miRNA from fresh frozen tissue.

Combined data from three cases of human colorectal cancer



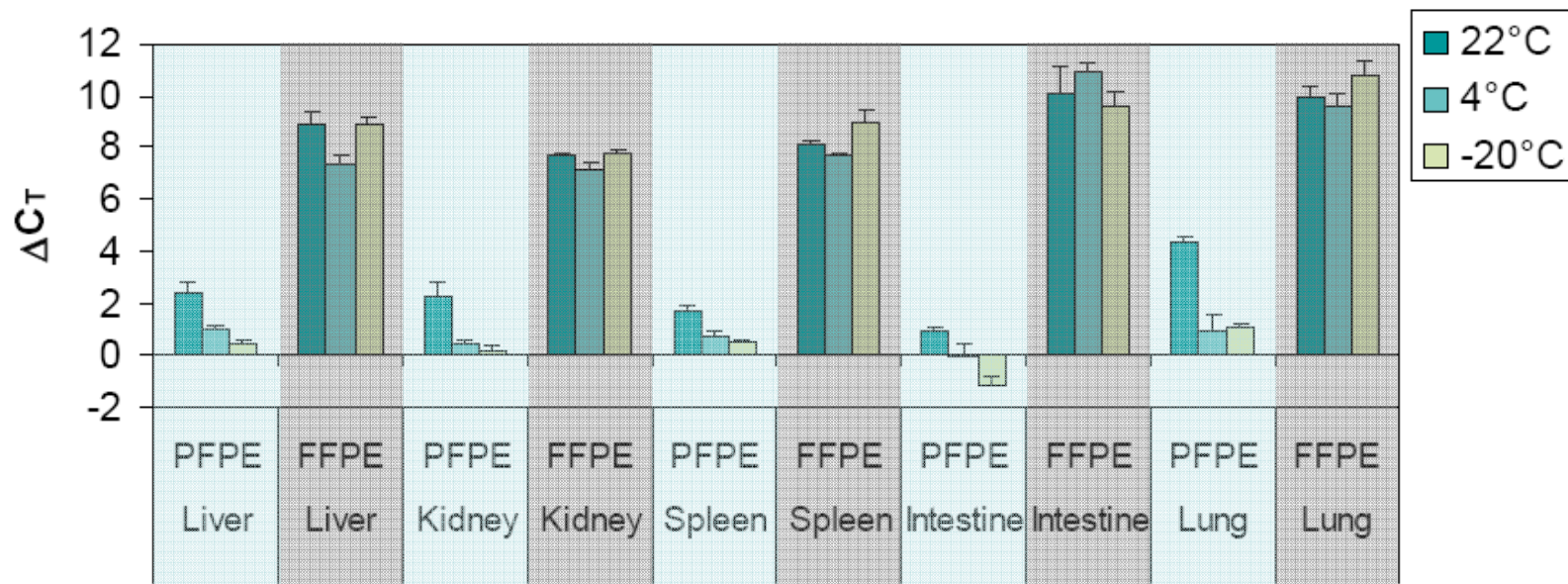
Viertler C, et al., J Mol Diagn. 2012 Sep;14(5):458-66.

PAXgene tissue can be stored for at least three years at with no adverse effect on morphology or RNA quality.



RNA from stored PFPE tissue performs as well as RNA from fresh frozen tissue in real time RT-PCR assays.

RT-qPCR of Rat β -Actin Gene: ΔC_T vs. Fresh Frozen (FF) Tissues



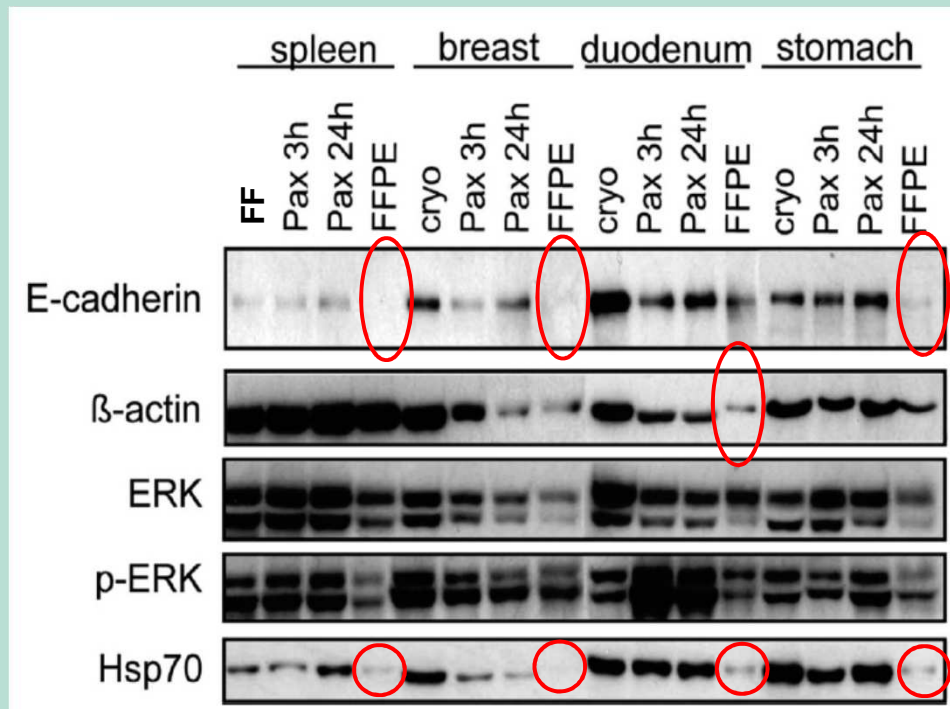
* $\Delta C_T = C_T(\text{RNA from PFPE or FFPE}) - C_T(\text{RNA from FF})$.

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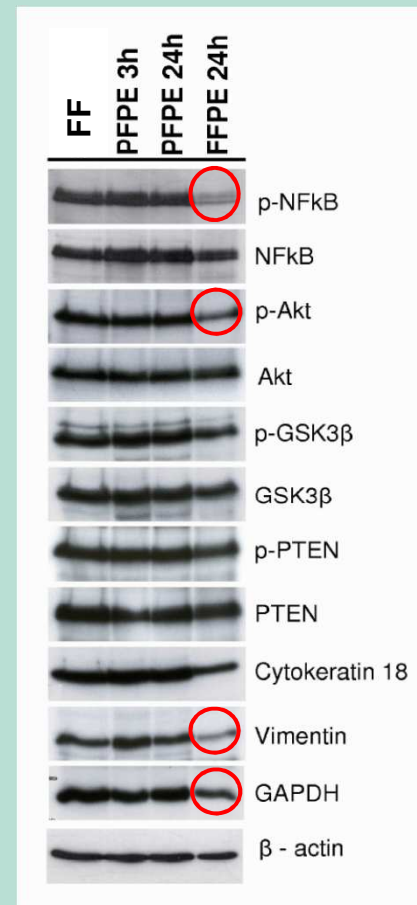
Proteins and phosphoproteins are preserved in PAXgene fixed tissue.

SDS-PAGE



Ergin et al. J Proteome Res. 2010 Oct 1;9(10):5188-96

Western Blot

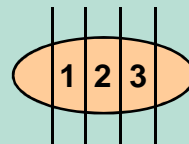


Viertler C, et al., J Mol Diagn. 2012 Sep;14(5):458-66.

Western blot analysis of phosphoproteins from PFPE samples: study design

Tissue types (n=20)	
Ovary	Ovarian cancer
Breast	Breast cancer
Prostate	Prostate cancer
Salivary gland	Bladder
Oesophagus	Gall bladder
Colon	Duodenum
Muscle	Tongue
Stomach	Thyroid gland
Kidney	Lung
Uterus	Pancreas

20 different tissue types



1 Frozen



2 PFPE



3 FFPE



Phosphorylation-specific antibodies (n=14)

Phospho-PTEN (Ser380)

Phospho-AKT (Ser473)

Phospho-GSK-3 β (Ser9)

Phospho-p38 MAPK (Thr180/Tyr182)

Phospho-HSP 27 (Ser78)

Phospho-p44/42 MAPK (Thr202/Tyr204)

Phospho-NF- κ B p65 (Ser536)

Phospho-STAT3 (Ser727)

Phospho-Tyrosin (total)

Phospho-Her2 (Tyr1248)

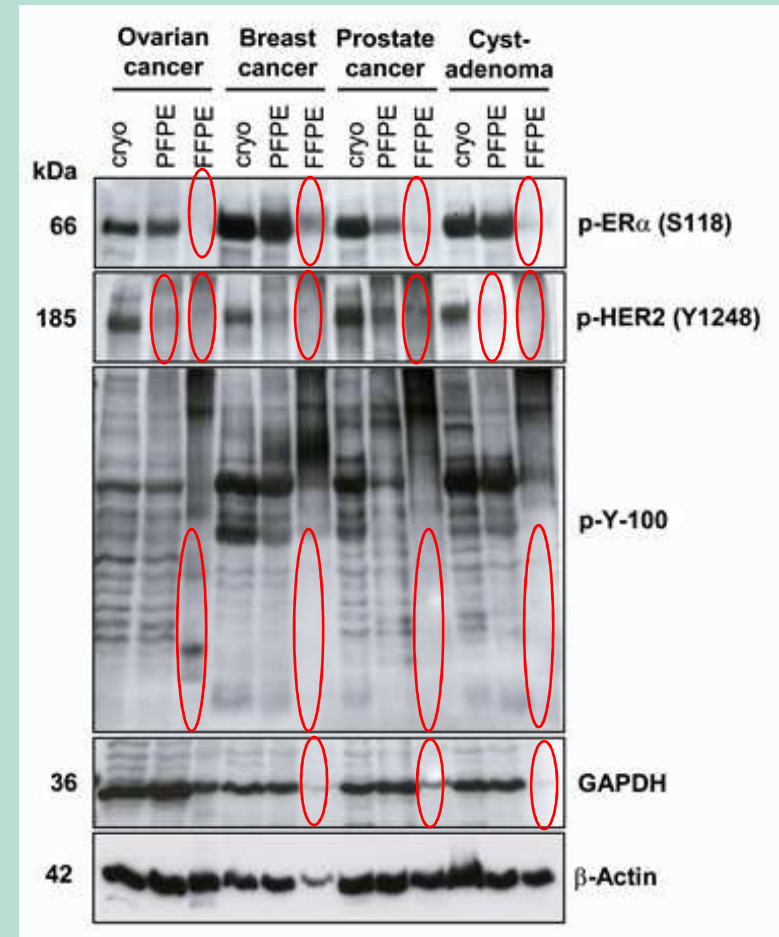
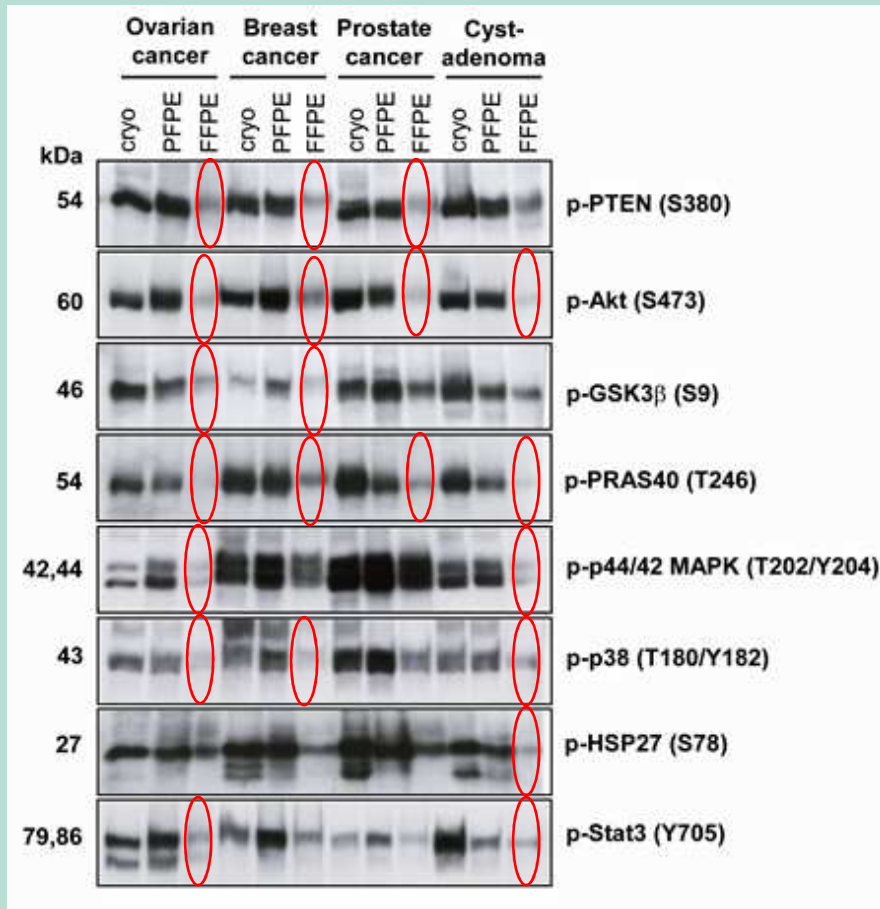
Phospho-Her3 (Tyr1289)

Phospho-ER α (Ser118)

Phospho-EGFR (Tyr1086)

Phospho-PGR (Ser190)

The phosphoproteome is quantitatively preserved in PFPE samples.

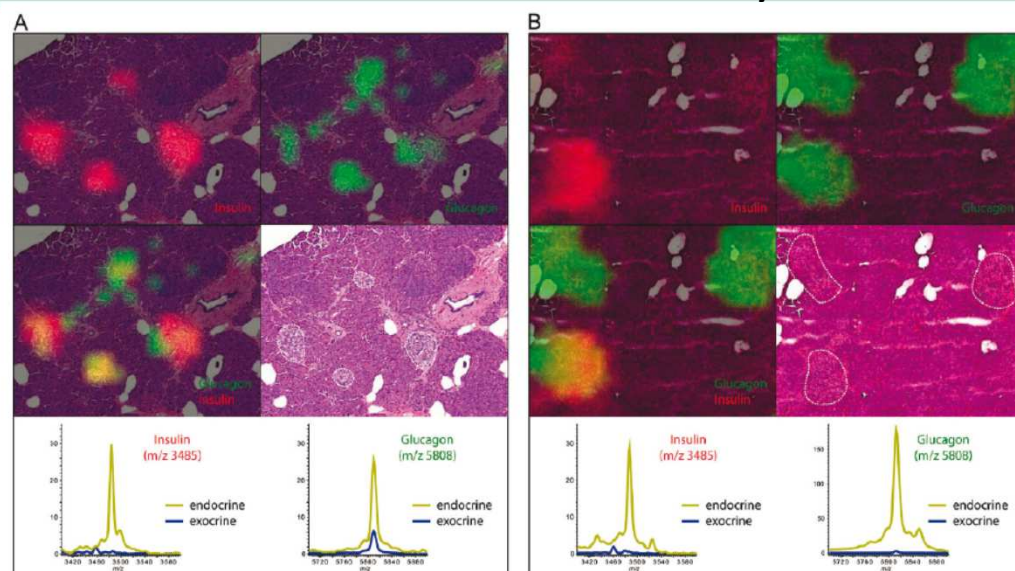
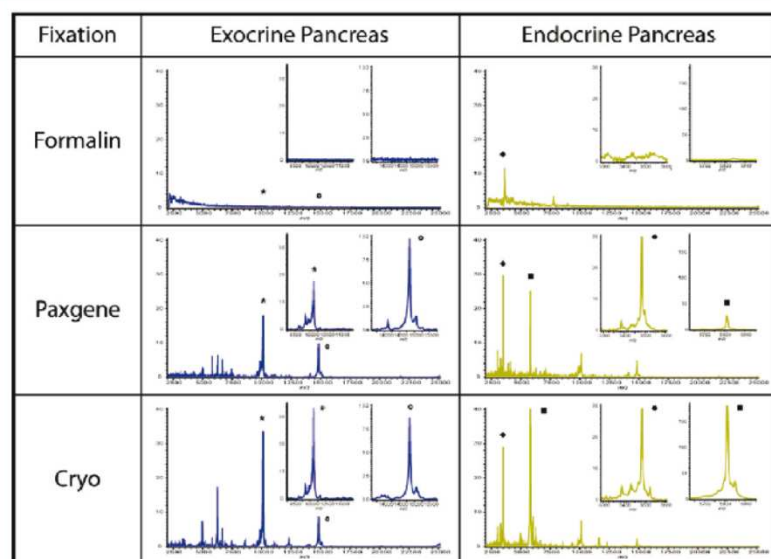


PAXgene fixation allows for the application of MALDI-IMS.

Pancreas

PFPE

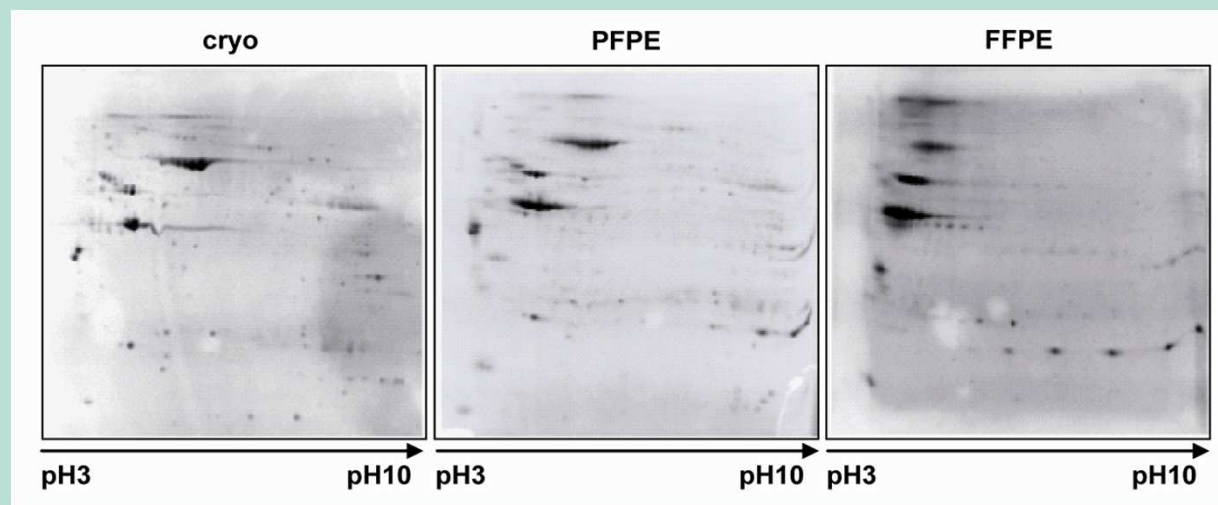
cryo



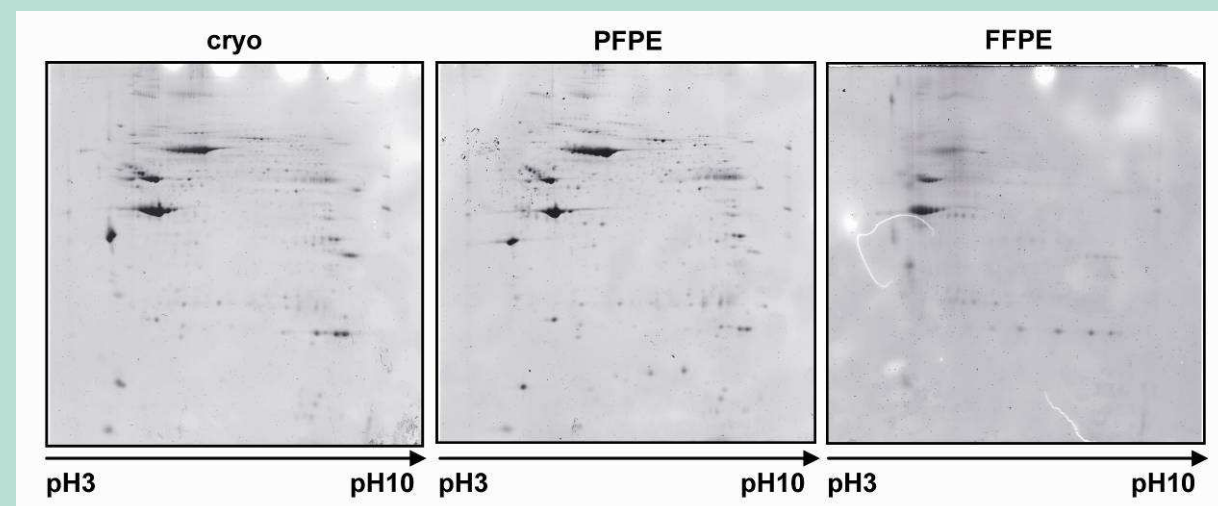
- In contrast to FFPE, PFPE and cryo-preserved pancreatic samples display a multitude of peaks
- Visualization of Insulin (m/z 3485) and Glucagon (m/z 5808) expression in pancreatic tissue.
- Spectra show the differential expression of Insulin and Glucagon comparing exocrine pancreas and endocrine pancreas

PFPE tissue is comparable to cryopreserved tissue in 2D-PAGE.

Colon



Duodenum



Summary of PAXgene tissue vs. FFPE and frozen tissue

The PAXgene Tissue System:

- ✓ **Simultaneously preserves histomorphology and biomolecules.**
 - Formalin preserves histomorphology, but not biomolecules.
 - Freezing preserves biomolecules, but not morphology.
- ✓ **Works without crosslinking or chemical modification.**
 - Formalin crosslinks biomolecules.
- ✓ **Allows for storage of tissue either in stabilization reagent or as paraffin blocks.**
- ✓ **Gives histology results comparable to FFPE while preserving biomolecules comparably to fresh freezing in liquid nitrogen.**

PAXgene Tissue enables combined histological and multimodal analysis of biomolecules from the same sample.

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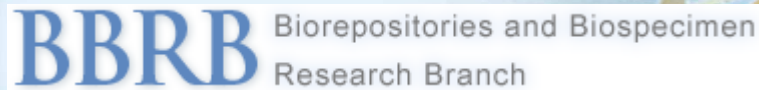
PAXgene performance has been proven in more than 10,000 human tissue samples.



Standardization and Improvement of Generic Pre-analytical Tools and Procedures for In Vitro Diagnostics

- European Commission grant EC FP7-HEALTH-2007-B
- Collaborative Research Project: 16 commercial and academic members
- Mission: To provide pan-European ***quality assurance schemes, guidelines*** and ***tools*** to minimize the effects of pre-analytical conditions on in vitro diagnostic results.

PAXgene has been chosen as the only fixative for the NIH/NCI GTEx program.



BBRB Biorepositories and Biospecimen
Research Branch

- The Genotype Tissue Expression Project (GTEx) is an NIH common fund initiative.
- Aims to establish a resource database and tissue bank in which to study the relationship between genetic variation and gene expression.
- Over 170 donors (>5000 tissue samples) have been collected to date.

"PAXgene is at least as good as formalin for histology. It is now our only morphologic fixative."

OBBR pathologists Philip Branton, John Madden James Robb, Leslie Sobin, Carolyn Compton (Critical Path Institute)

http://www.genome.gov/Pages/About/NACHGR/September2011AgendaDocs/NACHGR_Sep122011_%20GTExUpdate_Struewing.pdf

Acknowledgments

*QIAGEN GmbH

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- Leslie Sobin
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- Kristin Ardlie (Broad Institute)
- Carolyn Compton (Critical Path Institute)

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