Evaluation of the PAXgene® Tissue System: Preservation of Morphology and Gene Expression in Human Melanoma

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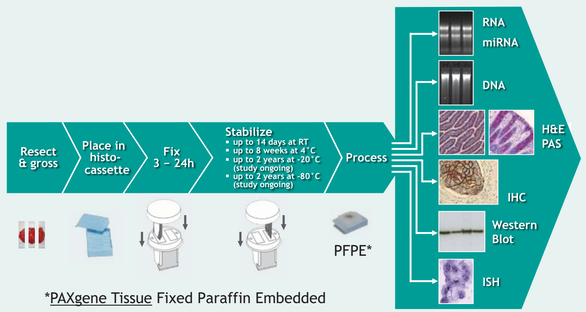
PreAnalytiX
A QIAGEN / BD Company

Introduction

PAXgene Tissue System

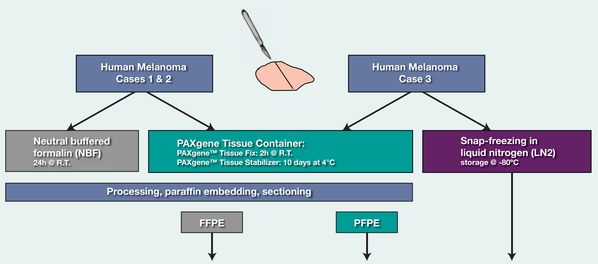
- Formalin-free tissue fixation method
- For simultaneous preservation of tissue morphology and biomolecules
- Allows analysis of biomarkers from the same sample used for histopathology
- Two reagent system for tissue fixation and stabilization
- Consists of the PAXgene Tissue Container, PAXgene Tissue Kits and supplementary protocols (www.preanalytix.com)
- Currently under evaluation by the EU FP7 funded project SPIDIA

PAXgene Tissue Workflow



*PAXgene Tissue Fixed Paraffin Embedded

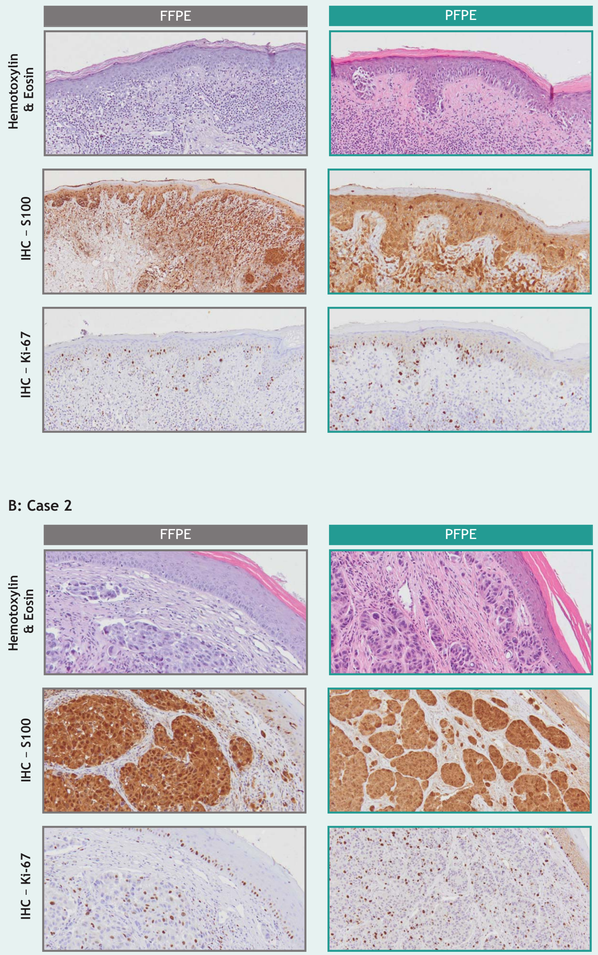
Materials and Methods



Staining	Hemotoxylin & Eosin, Immunohistochemical staining for Ki-67 and S100		–
RNA purification	RNeasy® FFPE (QIAGEN)	PAXgene Tissue RNA (PreAnalytiX)	RNeasy Mini (QIAGEN)
RNA incl. miRNA purification	miRNeasy® FFPE (QIAGEN)	PAXgene Tissue miRNA (PreAnalytiX)	miRNeasy Mini (QIAGEN)
RT-qPCR array	TaqMan® Array Gene Signature 96-Well Plate ´human endogeneous control´ (Applied Biosystems)		
miRNA specific RT-qPCR assays	TaqMan MicroRNA assays (Applied Biosystems), miScript Primer Assays and miScript PCR Control Set (QIAGEN)		

Results

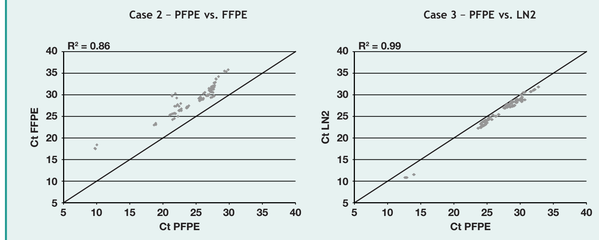
Figure 1: Staining



- Antibodies: Ki-67 clone MIB-1 (Dako M7240, 1:75 dilution); S100 polyclonal rabbit (Dako Z0311, 1:400 dilution)
- Epitope retrieval: FFPE 20 min at 98°C in citrate buffer pH6, PFPE 10 min at 70°C (Ki-67) or at 98°C (S100) in Tris/EDTA buffer pH9

Figure 2: RNA from Cases 1-3, Purity and Integrity

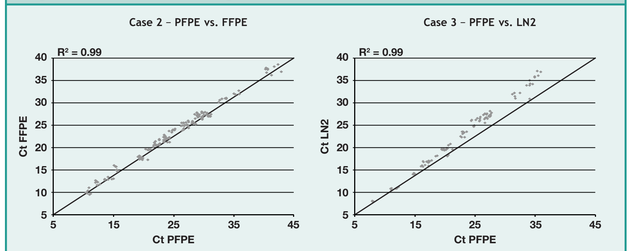
Figure 3: mRNA Profiling with RNA from Cases 2 and 3



Scatterplots for comparison of Ct values

- TaqMan Array: Human Endogenous Control with 32 control genes, plated in triplicates in a 96 well format
- Two Step RT-PCR with 2 µg RNA per plate
- R^2: coefficient of determination

Figure 4: miRNA Profiling with miRNA from Cases 2 and 3



Scatterplots with Ct values from 26 different noncoding small RNA specific RT-qPCR assays:

- 10x TaqMan MicroRNA Assays: miR10a, -16, -29a, -30b, -103, -137, -182, -192, let -7a, -7b
- 16x miScript miRNA Assays: miR9, -10a, -10b, -29a, -103, -125b, -143, -145, -155, -192
- 6x miScript PCR endogenous controls: RNUA1, RNU5A, RNU6B, SNORD25, SCARNA17, SNORA73A
- R^2: coefficient of determination

Summary

Stabilization of human melanoma tissue with PAXgene Tissue:

- H&E Morphology: well preserved, comparable to FFPE
- Antigenicity: for Ki-67 and S100 comparable to FFPE with regard to location, staining intensity and number of stained cells
- Gene expression profile:
  - high concordance between RNA from PFPE and snap frozen tissue
  - low concordance between RNA from PFPE and FFPE
- Small, noncoding RNA profiles:
  - high concordance between RNA/miRNA from PFPE and snap frozen tissue
  - high concordance between RNA/miRNA from PFPE and FFPE

Conclusions

This evaluation demonstrates the PAXgene Tissue System preserves morphology, antigenicity, RNA and miRNA profiles in human melanoma specimen