



Technical Note PAXgene[®] Tissue System

Detection of PI3K mutational status in DNA from human breast cancer PFPE tissue using the PI3K Mutation Test Kit (QIAGEN)

Introduction

Mutations in the PI3KCA gene are frequently found in human cancers. The PI3K Mutation Test Kit from QIAGEN detects 4 PI3K mutations (H1047R, E542K, E545K, and E545D) in exons 9 and 20 of the PIK3CA gene. The kit employs a real-time PCR assay based on ARMS[®] PCR technology combined with Scorpions[®] detection technology. A control assay is included in the kit to assess the total amount of amplifiable DNA in a sample. If a sample control C_T is ≤ 29 , the kit is capable of detecting as little as 1–2% mutated DNA in a background of wild-type genomic DNA (for further information about the PI3K Mutation Test Kit, see www.qiagen.com/products/PI3KMutationTestKit.aspx).

Study Design

Tumor samples from 10 cases of human breast cancer were used in this study. Samples from cases 1 to 5 were divided into 2 parts of approximately 4 x 10 x 10 mm in size. One part was fixed for 24 hours in neutral buffered formalin (NBF), and the other was fixed in PAXgene Tissue Fix in a PAXgene Tissue Container for 3 hours and stabilized for up to 3 days before processing. Samples from cases 6 to 10 were large enough to be divided into 3 parts. In addition to fixation with NBF and PAXgene Tissue Fix, the third part was snap-frozen in liquid nitrogen (LN2). NBF and PAXgene Tissue fixed samples were then processed and embedded in paraffin.

Sections with a thickness of 4 μ m were prepared from both PAXgene Tissue fixed, paraffin-embedded (PFPE) samples and from formalin-fixed, paraffin-

embedded (FFPE) samples. The samples were then stained with hematoxylin and eosin to analyze preservation of the morphology. DNA was purified from 3 x 10 µm sections of PFPE and FFPE tissue using the PAXgene Tissue DNA Kit and the QIAamp[®] DNA FFPE Tissue Kit (QIAGEN) respectively. DNA from 10 mg of snap-frozen tissue was purified using the QIAamp DNA Mini Kit (QIAGEN).

DNA yield and purity were analyzed spectrophotometrically by measuring the absorbance at 260 and 280 nm. The mutational status of the PIK3CA gene was analyzed using the PI3K Mutation Test Kit (QIAGEN) on an ABI PRISM[®] 7700 Sequence Detection System using 10 ng of DNA from each sample.

Results

Morphology was preserved in sections of FFPE and PFPE breast cancer samples after conventional hematoxylin and eosin staining (Figure 1).

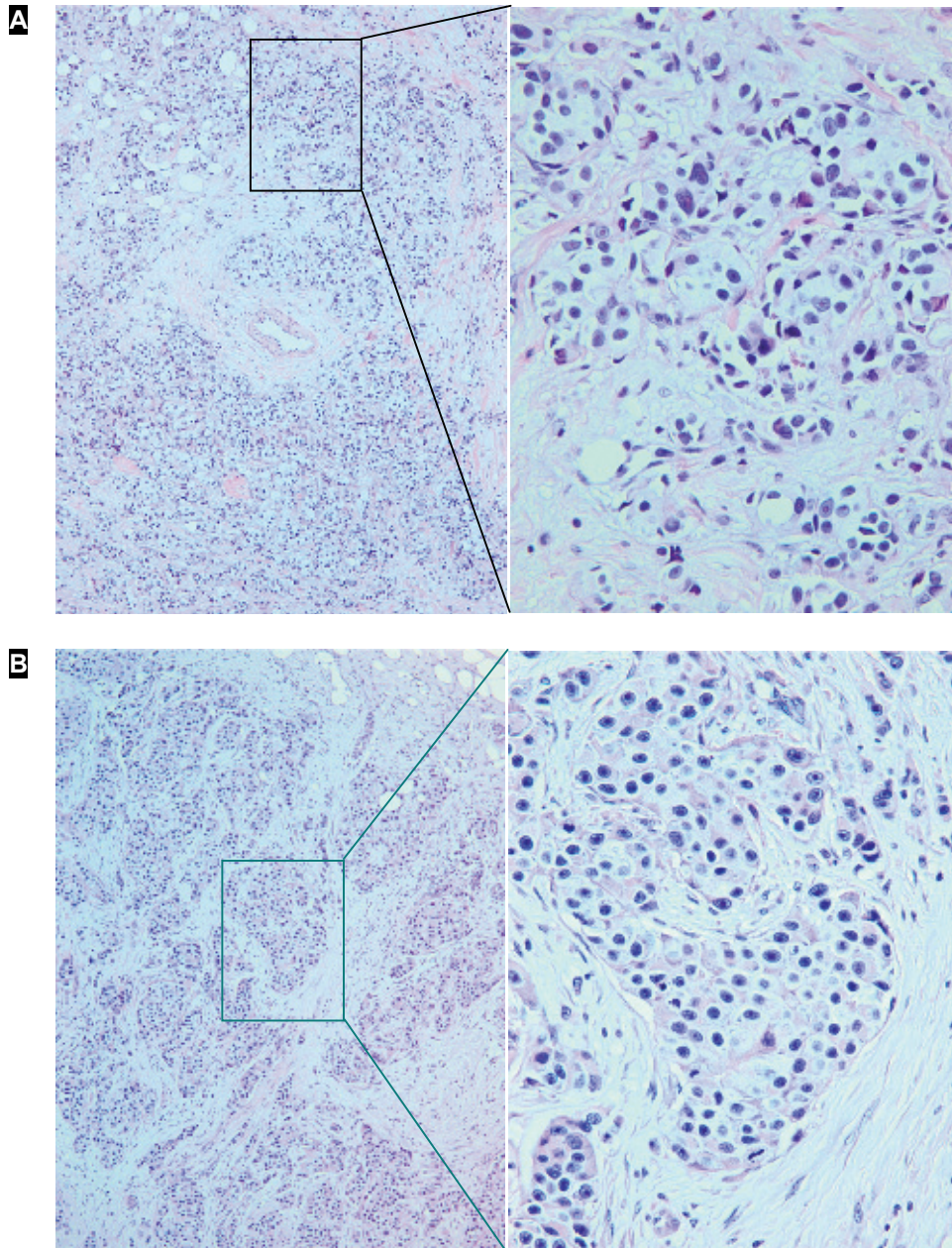


Figure 1. Preservation of morphology in PFPE and FFPE samples of human breast cancer. 4 μ m sections of **A** FFPE tissue and **B** PFPE tissue were stained with hematoxylin and eosin. Examples are shown from case 6 at 100x (left panel) and 400x (right panel) magnification.

The median DNA yields from 3 x 10 µm sections of PFPE and FFPE samples were 1.9 and 1.7 µg respectively (Figure 2). Median DNA yield from 10 mg of fresh frozen tissue was 5.8 µg (data not shown). Purity was generally high with median A_{260}/A_{280} values for all sample types between 1.8 and 1.9 (Figure 2).

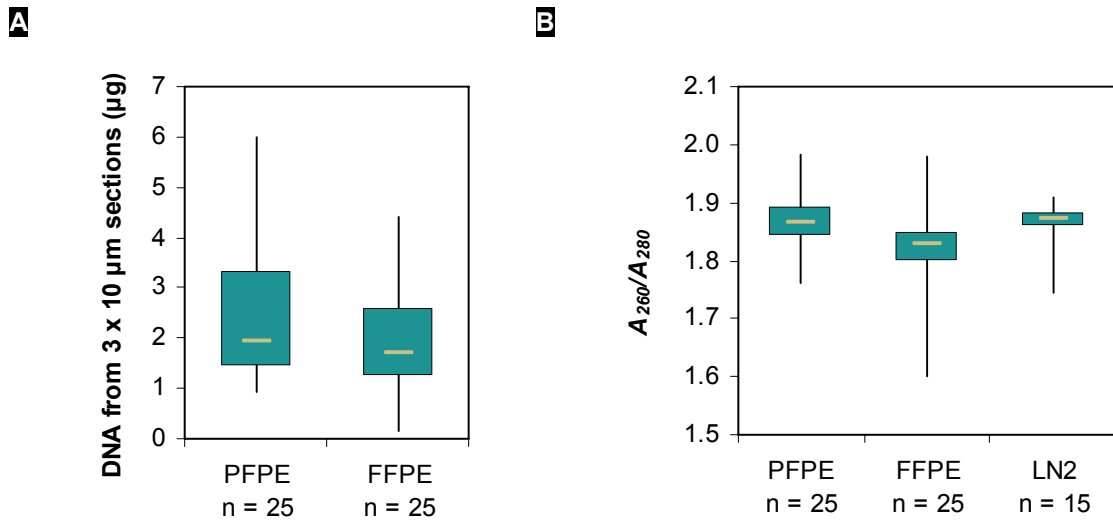


Figure 2. DNA yield and purity. Spectrophotometric analysis of **A** DNA yield and **B** DNA purity using a NanoDrop® instrument. DNA was purified from 3 x 10 µm sections of mirrored **PFPE** and **FFPE** samples, in duplicate for cases 1–5 and in triplicate for cases 6–10. In addition DNA was purified in triplicate from 10 mg tissue fixed in liquid nitrogen (**LN2**) for cases 6–10. Box plots show median, lower, and upper quartiles as well as smallest and largest observed values (sample minimum and maximum).

Gel electrophoresis showed that DNA isolated from snap-frozen and PFPE tissue samples was of high molecular weight, appearing as a distinct band at approximately 10 to 20 kb. In contrast, DNA from FFPE tissues did not run as a distinct band but as a smear (Figure 3).

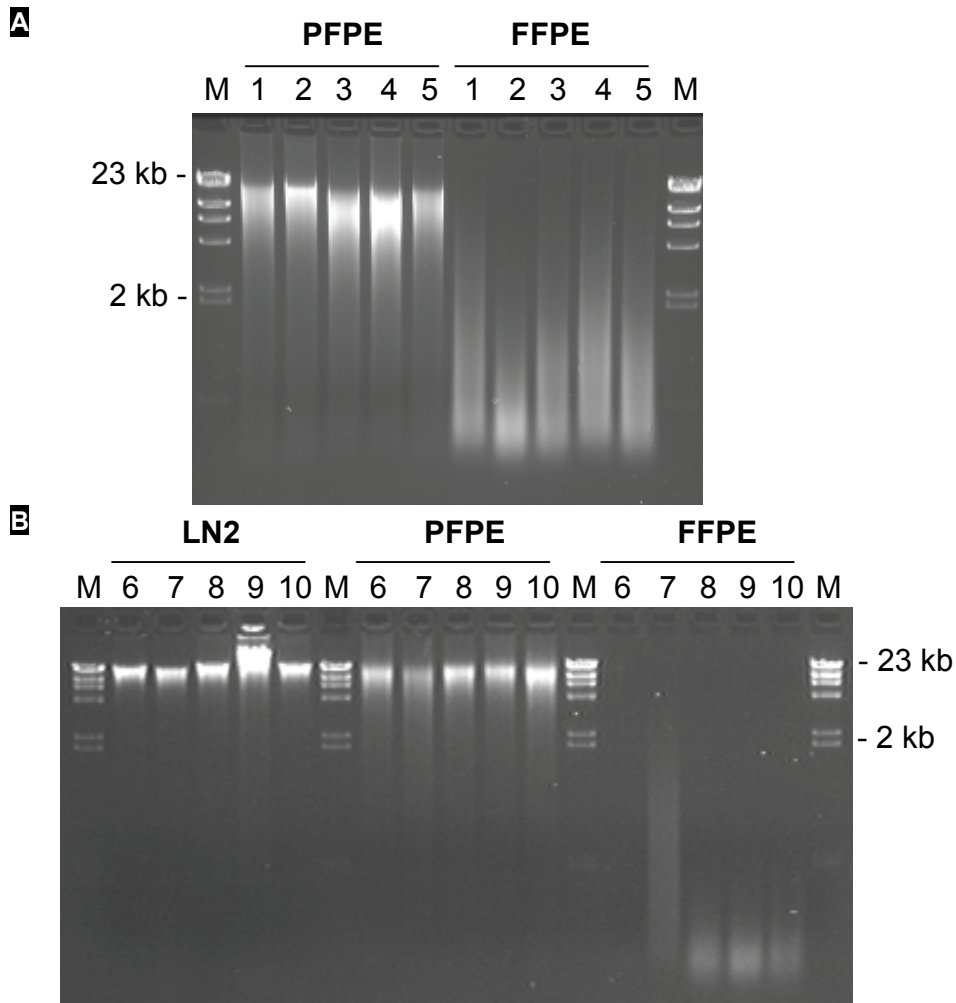


Figure 3. DNA integrity. Gel electrophoresis with genomic DNA from 10 cases of breast cancer: **A** cases 1–5, mirrored samples of PFPE and FFPE tissue, and **B** cases 6–10, mirrored samples snap-frozen in liquid nitrogen (LN2), PFPE tissue, and FFPE tissue. **M**: lambda *Hind* III marker, with 23 kb and 2 kb bands indicated.

Mutational status of the most common mutations in the PIK3CA gene was determined by real-time PCR using the PI3K Mutation Test Kit (QIAGEN). Each case gave identical results, regardless of the tissue fixation or DNA purification method (Table 1).

Table 1. Mutational status of PI3K mutations in exons 9 and 20 in DNA from 10 cases of human breast cancer

Sample	ΔC_T ($C_T^{\text{mutation-specific assay}} - C_T^{\text{control assay}}$)	1% ΔC_T (if control $C_T \leq 29$)	Mutation status
Case 3 — PFPE	2.4	7.2	Positive H1047R
Case 3 — FFPE	2.8	7.2	Positive H1047R
Case 4 — PFPE	6.1	7.7	Positive E545K/D
Case 4 — FFPE	6.3	7.7	Positive E545K/D
Case 5 — PFPE	3.8	7.2	Positive H1047R
Case 5 — FFPE	3.9	7.2	Positive H1047R
Cases 1, 2, 6–10	–	–	Negative

The PI3K Kit control assay, which amplifies a conserved region of exon 15 of the PIK3CA gene, is used to assess the total yield of amplifiable DNA in a sample. C_T values for the control assay using 10 ng of DNA from PFPE or snap-frozen samples were consistently between 25 and 27, below the threshold of 29 specified for maximum sensitivity of 1–2% mutation detection in a background of wild-type DNA. In contrast, C_T values for the control assay using 10 ng DNA from FFPE tissue samples ranged from 27 to 37, indicating that, in some samples, only higher levels of mutations are detected (Figure 4).

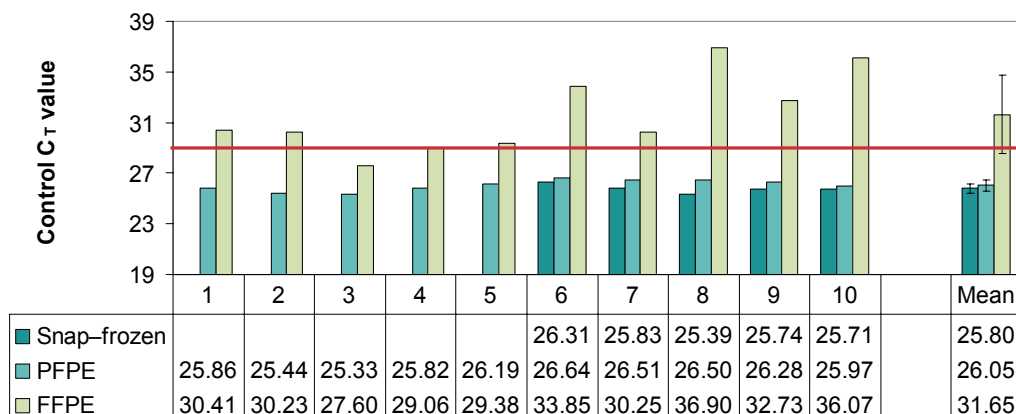


Figure 4. C_T values obtained from 10 ng DNA using the PI3K Test Kit control assay. Genomic DNA from 10 cases of breast cancer: cases 1–5, mirrored samples of PFPE and FFPE tissue, and cases 6–10, mirrored samples snap-frozen in liquid nitrogen (LN2), PFPE tissue, and FFPE tissue. The threshold C_T value of 29 is marked with a red line. **Mean:** Mean C_T value.

Conclusions

Results of this study indicated that DNA isolated from human breast cancer PFPE tissues using the PAXgene Tissue DNA Kit is of high purity and high molecular weight. DNA from PFPE samples available for this study could be successfully used for detection of mutations in the PIK3CA gene using the PI3K Mutation Test Kit from QIAGEN. Sensitivity was similar to that attained when using DNA from fresh frozen tissue samples.

PAXgene Tissue System and PI3K Mutation Test Kit: For research use only. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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