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Technical Note
PAXgene® Tissue System

Quantitative analysis of KRAS and BRAF mutational status in DNA from PAXgene Tissue fixed, paraffin-embedded (PFPE) tissue using Pyrosequencing® technology

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Study Design

Tumor samples from 5 cases of human colorectal cancer (CRC) were divided into parts of approximately 4 x 10 x 10 mm. One part was snap frozen in liquid nitrogen and another part was placed into a standard tissue cassette for fixation within the PAXgene Tissue Container for 2–4 hours and stabilization for 3 days. Processing and paraffin embedding were performed according to recommendations in the *PAXgene Tissue Container Product Circular*.

Sections of 4 µm were prepared from PAXgene Tissue fixed, paraffin embedded (PFPE) samples and were stained with hematoxylin and eosin to analyze morphology preservation. DNA was purified from three 10 µm sections of PFPE tissue using the PAXgene Tissue DNA Kit and from 10 mg of snap-frozen tissue using the QIAamp® DNA Mini Kit. All extractions were performed in triplicate. DNA yield and purity were analyzed spectrophotometrically by measuring the absorbance at 260 and 280 nm. Integrity was analyzed by agarose gel electrophoresis. Mutational status of KRAS codons 12, 13, and 61 and BRAF codon 600 was determined by Pyrosequencing on a PyroMark® Q24 MDx* using the KRAS Pyro® Kit and the BRAF Pyro Kit.

* Not available in the United States or Canada.

Results

Sections of PFPE CRC samples were used successfully for conventional hematoxylin and eosin staining (Figure 1). In addition, DNA isolated from PFPE samples was of high molecular weight, appearing as a distinct band at approximately 20 kb (Figure 2). DNA yield from three 10 µm PFPE sections was 1.8–9.3 µg. The $A_{260/280}$ absorbance was 1.76–1.87, indicating the quality of the DNA is high.

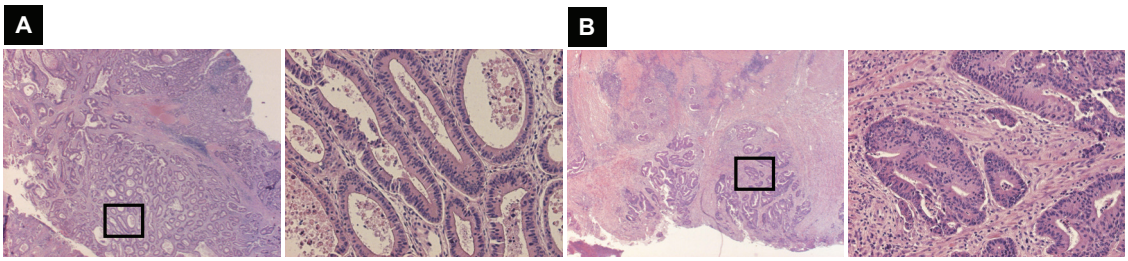

Figure 1. Morphology of CRC from PFPE samples. Hematoxylin and eosin staining of 4 µm sections. Examples are shown for A case 1 and B case 2 at magnifications of 12.5x and 100x, respectively.

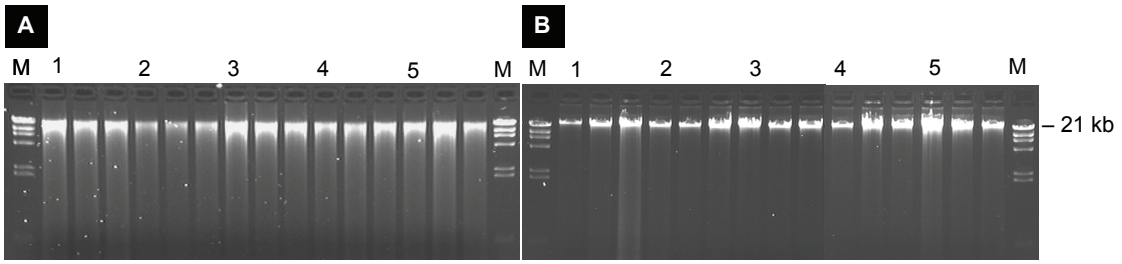

Figure 2. DNA integrity. Samples were prepared and DNA was purified as described in "Study Design." Gel electrophoresis was performed on 0.8 % TBE-buffered agarose gels with 200 ng genomic DNA from 5 different CRC cases (1–5). A DNA from sections of PFPE tissue. B DNA from snap-frozen tissue. M: lambda *Hin*d III marker, with 21 kb band indicated.

Mutational status of KRAS and BRAF was successfully determined for all samples. No differences were found in DNA from snap-frozen and PFPE samples. A mutation in KRAS codon 13 (GAC) was detected for case 2, and a mutation in BRAF codon 600 (GAG) was detected for case 3 (Figure 3 and Table 1).

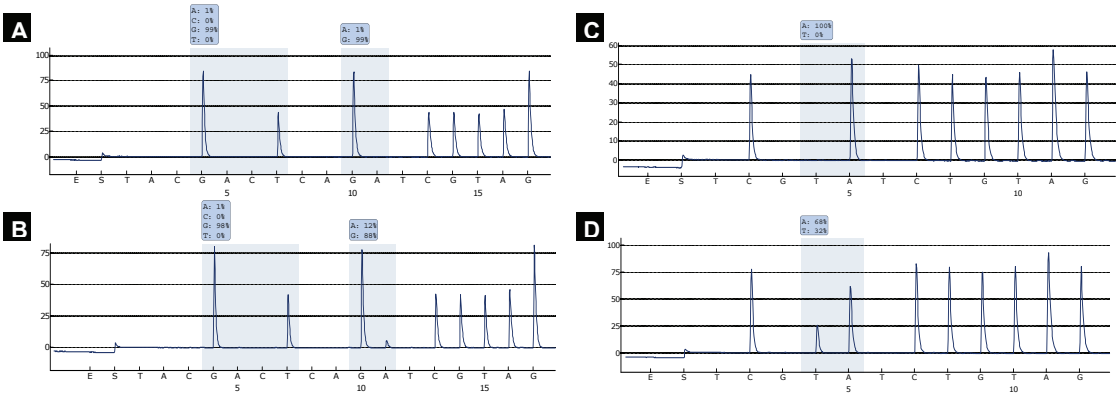

Figure 3. Typical pyrograms obtained using the codon 12/13 assay (A and B) of the KRAS Pyro Kit and the codon 600 assay (C and D) of the BRAF Pyro Kit. Human genomic DNA isolated from sections of PFPE tissue was amplified using the HotStarTaq® Plus Mastermix Kit and was analyzed using Pyrosequencing technology with the corresponding sequencing primers. Pyrograms® were obtained using A DNA from case 1, which exhibits wildtype (WT) sequence for KRAS codon 13, B DNA from case 2, which exhibits a 12% GGC→GAC mutational level for codon 13, C DNA from case 1, which exhibits WT sequence for BRAF codon 600, D DNA from case 3, which exhibits a 32% GTG→GAG mutational level of codon 600 (assayed in reverse orientation).

Table 1. Mutational status of DNA from 5 CRC samples

	KRAS			BRAF
Case	**Codon 12**	**Codon 13**	**Codon 61**	**Codon 600**
1	GGT (WT)	GGC (WT)	GTT (WT)	GTG (WT)
2	GGT (WT)	GAC (mutant)	GTT (WT)	GTG (WT)
3	GGT (WT)	GGC (WT)	GTT (WT)	GAG (mutant)
4	GGT (WT)	GGC (WT)	GTT (WT)	GTG (WT)
5	GGT (WT)	GGC (WT)	GTT (WT)	GTG (WT)

Conclusion

Samples of human CRC tissue were fixed with the PAXgene Tissue Container for preservation of tissue morphology and DNA purification. High-molecular-weight DNA was isolated from PFPE tissues with the PAXgene Tissue DNA Kit. The DNA was suitable for use in Pyrosequencing.

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX® or QIAGEN® kit handbook or user manual. PreAnalytiX and QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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