PreAnalytiX
A QIAGEN / BD Company

Detection of Mitochondrial DNA in DNA Purified Using the PAXgene™ Blood DNA System

Mitochondrial DNA (mtDNA) is often used in studies of human evolution and gene inheritance. Furthermore, several systemic diseases are associated with mutations in mitochondrial genes, such as Leber's hereditary optic neuropathy (a form of blindness) and Leigh disease (a neurodegenerative disorder). Here we show that DNA isolated using the PAXgene™ Blood DNA System contains mitochondrial DNA that can be analyzed by Southern blot analysis or PCR amplification.

Experimental design: Human whole blood samples from 8 donors were drawn into PAXgene Blood DNA Tubes, and DNA was purified using the PAXgene Blood DNA Kit according to the standard protocol. Each DNA sample was dissolved in 1 ml Buffer BG4 (resuspension buffer).

Results: Using the QIAGEN® Multiplex PCR Kit, three mitochondrial gene fragments were amplified in one assay (Figure 1): 922 bp from the $tRNA^{lys}$/ATPase gene, 629 bp from the $tRNA^{leu(UUR)}$ gene, and 293 bp from the ND_4 gene (1). The 922 bp fragment was used as a digoxygenin-labeled probe in a Southern blotting experiment. For Southern blotting, 5 µg DNA from 3 of the 8 donors was digested with EcoRI, separated on an agarose gel, blotted onto a nylon membrane, and hybridized with the probe. EcoRI cuts the circular 16.6 kb mitochondrial genome at 3 sites to create fragments of 1.1, 7.4, and 8.1 kb. The 922 bp fragment of the $tRNA^{lys}$/ATPase gene hybridized with the 7.4 kb fragment (Figure 2). No other hybridization signals could be detected, indicating complete digestion of the DNA samples by EcoRI.

Multiplex PCR of 3 Mitochondrial Gene Fragments

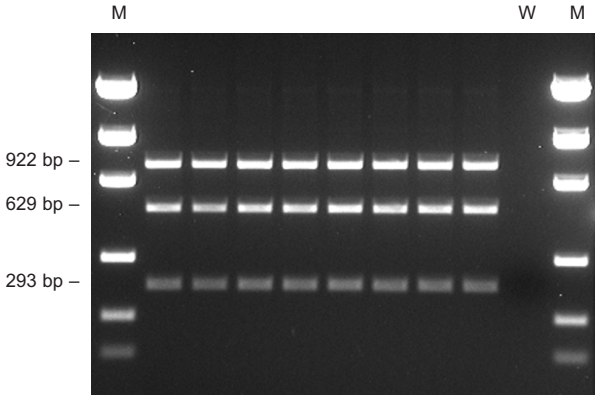

Figure 1. *Multiplex PCR of fragments from the mitochondrial genes $tRNA^{lys}$/ATPase (922 bp), $tRNA^{leu(UUR)}$ (629 bp), and ND_4 (293 bp), using 250 ng DNA from 8 donors as starting material.* ***M****: markers;* ***W****: water control.*

Conclusion: DNA purified using the PAXgene Blood DNA System can be used in assays for the detection of mitochondrial DNA sequences.

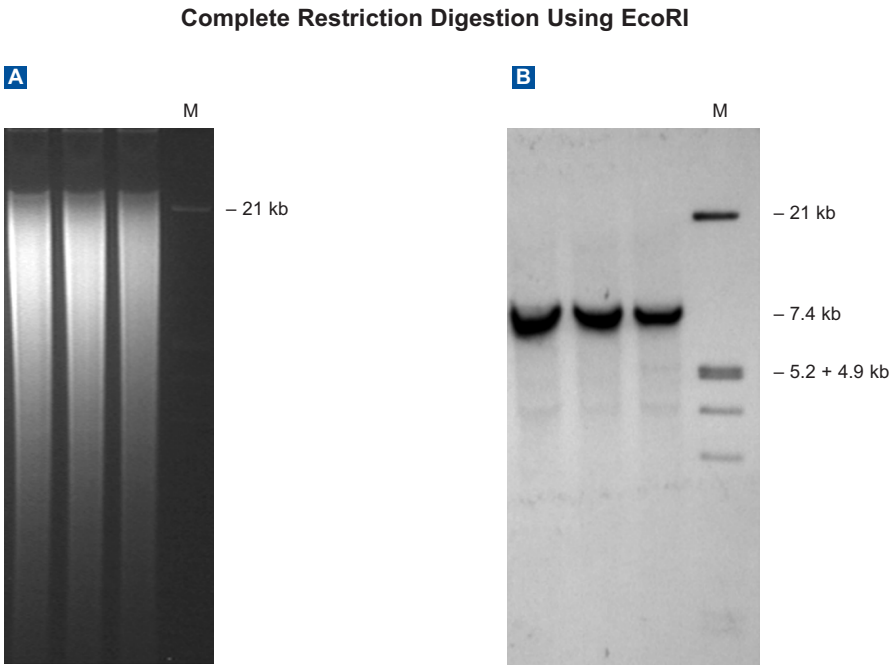

Figure 2. **A** *Agarose gel analysis of 5 µg DNA restriction-digested by EcoRI (0.8% agarose gel, 1x TAE buffer, 23 V, 16 h).* **B** *Southern blotting using a digoxygenin-labeled 922 bp fragment of the mitochondrial $tRNA^{lys}$/ATPase gene.* ***M****: marker.*

1. Wong, L.J., and Senadheera, D. (1997) Direct detection of multiple point mutations in mitochondrial DNA. Clin. Chem. **43**, 1857.