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Protein Truncation Testing on the DCode™ System

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Introduction

Protein Truncation Testing (PTT) is a method used to screen large coding regions of DNA to detect translation termination mutations. The gene responsible for the autosomal recessive disease ataxia-telangiectasia, ATM (ataxia-telangiectasia, mutated),[1] is a large transcript, approximately 10 kb, which lends itself well to PTT by analysis of seven overlapping regions (a–g) for truncated in vitro translation products.[2] Here, we report our PTT analysis of ATM mutations. Approximately 80% result in truncation.

Method

Twelve samples were RT-PCR* amplified for region b of ATM using specialized PTT modified primers containing a T7 promoter and a eukaryotic translation initiation sequence. One hundred nanograms of amplified product was used to produce protein in the coupled transcription-translation reaction of the TNT Coupled Reticulocyte Lysate System (Promega). Reactions were performed in 12.5 ml with 6 µCi of ^{35}S-methionine. Protein products were run on a 16 x 20 cm, 14% SDS-PAGE denaturing polyacrylamide gel using the DCode universal mutation detection system. The samples were run at room temperature in 1x Tris/Glycine/SDS buffer (25 mM Tris, 192 mM glycine, 0.1% SDS) at 200 V for 3 hours. The gel was fixed for 15 minutes, washed in Amplify (Amersham Life Science) for an additional 15 minutes, dried and exposed to film (Kodak X-OMAT-AR) overnight.

Results and Discussion

Region b amplification of 12 different AT patient samples resulted in a 1,300 bp product which encoded a 45 kD protein. PTT analysis of the 12 samples identified two samples having premature termination mutations. These truncated proteins were detected by having a different migration pattern compared to the full-length protein product when run on an SDS-PAGE using the DCode system (Figure 1).

Lane 2 shows a protein product smaller than the normal protein. The fact that no normal product is observed in this lane usually indicates a homozygous mutation. Sequencing of cDNA indicated a deletion of 174 bp, corresponding to the entire exon 26. Because this cDNA mutation would result in an in-frame deletion, the final protein product would lack only 58 amino acids. Genomic sequencing analysis revealed a homozygous G to A substitution at the last nucleotide of exon 26 (nt 3576). This mutation is referred to as 3576 G > A (c1135del174nt).

Sample 10 showed the presence of two protein products, one of normal length and a second of smaller size, suggesting this patient to be heterozygous for a mutation in this region. Sequence analysis identified a nonsense mutation at 5971 nt substituting a G to T (5971G>T[c1990]).

PTT analysis has proven to be advantageous in initial attempts to screen for mutations in a large gene such as ATM. This method allows for detection of any out-of-frame insertions or deletions resulting in a truncated protein, as well as for substitutions creating a nonsense mutation. PTT will also reveal large in-frame insertions or deletions producing a gross protein product alteration.

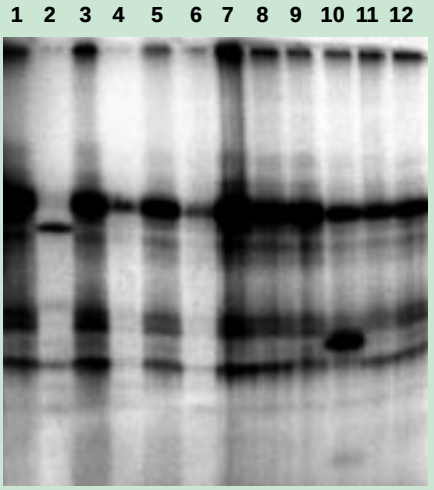

Fig. 1. PTT samples run on SDS-PAGE on the DCode system. Lanes 1–12 show PTT products of ATM region b for 12 different patient samples. Lanes 2 and 10 show truncated proteins.

Summary

The versatile DCode system can be used for multiple mutation detection techniques, including single-stranded conformational polymorphism (SSCP), conformation-sensitive gel electrophoresis (CSGE) and denaturing gradient gel electrophoresis (DGGE). Protein truncation test analysis can also be performed on the DCode system, producing results equivalent to other electrophoretic systems.

References

1. Savitsky, K., *et al.*, *Science*, **268**, 1749-1753 (1995).

2. Telatar, M., *et al.*, *Am. J. Hum. Genet.*, **59**, 40-44 (1996).

*The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffmann-LaRoche. Use of the PCR process requires a license.

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