B1 Alternative DNA Structures and Their Effect on Polymerase Activity in Polymerase Chain Reactions

Julie L. Langdon, MS, and Stephen A. Winkle, PhD, Florida International University, 8th Street, Miami, FL*

The goals of this presentation are to emphasize the importance of thoroughly researching the STR loci chosen for forensic DNA profiling.

This presentation will provide evidence that incorrect products can be obtained by Polymerase Chain Reaction when amplifying regions that have the potential of containing alternate DNA structures.

Polymerase Chain Reaction (PCR) is an enzymatic method currently used to amplify the Short Tandem Repeat (STR) loci used in forensics. Research has found that alternate DNA structures can modify polymerase activity. This is important for forensics because repeating DNA has been implicated in the formation of alternate DNA structures. To further investigate the effects of alternate DNA structure on polymerase activity, this study investigated the polymerase activity during PCR of a potential cruciform region and an alternate $(GC)_3$ region in the φX174 bacteriophage.

PCR products obtained by using various extension temperatures or magnesium concentrations were investigated using polyacrylimide gel electrophoresis, denaturing gel electrophoresis, and restriction enzyme digestion. Extension temperature and magnesium concentration was chosen to study because both factors can directly affect the polymerase activity during PCR. The results showed an increase in the amplification yield of the cruciform region when using a lower extension temperature (52ºC), while producing a very low amplification yield at the standard 72ºC extension temperature. The study also indicated the presence of stutter in the $(GC)_3$ region as the magnesium concentration increased. Prior to PCR, linearization of φX174 was completed to alleviate negative supercoiling, which is necessary for extrusion of the alternate DNA structures. The amplification of the cruciform region improved suggesting the presence of an alternate DNA structure reduced amplification prior to linearization. *Hha*I digestion of the cruciform region PCR product was altered signifying that the local sequence induces a structural deviation even in the absence of negative supercoiling.

In conclusion, this study demonstrated that Polymerase Chain Reactions are influenced by the presence of alternate DNA structures in the template DNA. The study also suggested that the formation of alternate DNA structures is influenced by the local DNA sequence even in the absence of negative supercoiling, therefore suggesting that even after PCR alterations in the amplified product may exist. These results suggest that the fidelity of forensically relevant STR loci may be affected if the loci have the ability to contain alternate DNA structure. The presence of an alternate DNA structure in the loci may cause a variation in the polymerase activity during PCR and therefore may inadvertently cause incorrect DNA profiling. This suggests the need to study the loci presently used or any loci developed to eliminate the possibility that the loci may exist as alternate DNA structures.

PCR, Alternate DNA Structures, STRs

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