## Enzymes

### 21 Direct and indirect quantification of polymerase chain reaction products with a time-resolved fluorometric scanner

**Chan, A.**, Krajden, M., and Diamandis, E.P.
Departments of Clinical Biochemistry and Microbiology, The Toronto Hospital, Toronto Western Division, 399 Bathurst Street, Toronto, Ontario, M5T 2S8, Canada

The polymerase chain reaction (PCR) is widely used in research but is still a qualitative tool. Recently, there has been increased interest in quantifying the PCR products especially for clinical applications, e.g., in detecting viremia and in measuring the *in vivo* effects of antiviral agents. Thus, reliable, sensitive and non-isotopic methods to detect PCR product quantity and size are necessary. We devised new methods which allow direct quantification of PCR products in agarose gels, and indirect quantification on nylon membranes or in microtiter wells. In the first method, one of the PCR primers was synthesized to contain a free amino group in the 5' end. This amino group was used to label the primer with the europium chelator 4,7-bis (chlorosulfophenyl) 1,10-phenanthroline 2,9-dicarboxylic acid (BCPDA). After PCR and agarose gel electrophoresis, the gel is immersed in a $EuCl_3$ solution for 30 min. The gel is then washed briefly and scanned with the Cyberfluor 615™ immunoanalyzer/scanner (a time-resolved fluorometer) to quantify the fluorescent $Eu^{3+}$-BCPDA complex. This method was tested succesfully by using as target a cytomegalovirus DNA sequence which was cloned in a plasmid. Similarly, we quantified the product after transferring the fluorescent band on a nylon membrane by the Southern blot technique. We have also cut the fluorescent band from the gel, digested agarose enzymatically and measured fluorescence in microtiter wells. The same direct and indirect methods were used to quantify PCR products with unlabelled primers and BCPDA-labelled nucleotides. These novel methodologies offer the advantages of sensitivity, simplicity and speed.