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DEOXYNUCLEOTIDE TRIPHOSPHATES (dNTPs) CONCENTRATION IN POLYMERASE CHAIN REACTION: EFFECTS ON DNA AMPLIFICATION

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ABSTRACT

DEOXYNUCLEOTIDE TRIPHOSPHATES (dNTPs) CONCENTRATION IN POLYMERASE CHAIN REACTION: EFFECTS ON DNA AMPLIFICATION

The roles of various reagents in different polymerase chain reaction (PCR) protocols and the necessity to optimize specific PCR applications with respect to deoxynucleotide triphosphates (dNTPs) have been discussed by many researchers. In this study, the concentration of dNTPs influencing PCR amplification was carried out. DNA amplification was performed using six different concentration of dNTPs with other PCR reagents including the *Taq* polymerase, primer and DNA template concentrations, the PCR cycling parameters and the number of PCR cycles remained constant. The amplified DNA were separated by agarose gel electrophoresis and stained with ethidium bromide. The visualization of nucleic acid band was done using a gel documentation system. The results indicated that the dNTPs is needed in the amplification of DNA. DNA amplification for this particular DNA template and PCR profile was optimal with 0.08 mM dNTPs concentration. A further increased or decreased of the dNTPs concentration results in poor amplification of DNA. In concentration of 0.02 mM, DNA amplification still occurred but in the absence of dNTPs, there was no amplification produced. In the excess of 0.10 mM dNTPs, the band failed to form. In conclusion, the concentration of dNTPs has significant effects on the amplification of DNA. Excessively high or low dNTPs concentration will result in a corresponding low yield or no amplification of DNA at all. The dNTPs concentration must therefore be determined empirically in order to yield good DNA amplification.

CHAPTER 1

INTRODUCTION

1.1 Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a relatively simple technique that amplifies a DNA template to produce specific DNA fragments *in vitro*. Traditional methods of cloning a DNA sequence into a vector and replicating it in a living cell often require days or weeks of work, but amplification of DNA sequences by PCR requires only hours (Sambrook and Russell, 2001). While most biochemical analyses, including nucleic acid detection with radioisotopes, require the input of significant amounts of biological material, the PCR process requires very little. Thus, PCR can achieve more sensitive detection and higher levels of amplification of specific sequences in less time than previously used methods (Weissensteiner, *et al.*, 2004). These features make the technique extremely useful, not only in basic research, but also in commercial uses, including genetic identity testing, forensics, industrial quality control and *in vitro* diagnostics. Basic PCR has become a commonplace in many molecular biology laboratories where it is used to amplify DNA fragments and detect DNA or RNA sequences within a cell or environment. However, PCR has evolved far beyond simple amplification and detection, and many extensions of the original PCR method have been described.