UNIVERSITI TEKNOLOGI MARA

OPTIMIZING THE MAGNESIUM CHLORIDE CONCENTRATION IN A POLYMERASE CHAIN REACTION

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Project submitted in fulfillment of the requirement for the degree of Bachelor of Science (Hons.) Medical Technology

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This Final Year Project Report entitled " **Optimizing Magnesium Chloride Concentration in Polymerase Chain Reaction (PCR)**" was submitted by Sazaliza Bt Mat Isa, in partial fulfillment of the requirement for the Degree of Bachelor of Science (Hons.) Medical Technology in the Faculty of Health Science, and was approved by

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ABSTRACT

OPTIMIZING THE MAGNESIUM CHLORIDE CONCENTRATION IN A POLYMERASE CHAIN REACTION

The polymerase chain reaction (PCR) is any enzymatic method of synthesizing or amplification of large quantities of a targeted region of DNA *in vitro*. When developing a protocol for PCR amplification of a new DNA target, it may be important to optimize the reagent concentrations, cycling temperatures and cycle numbers. Magnesium chloride ($MgCl_2$) had been shown to influence the primer annealing temperature, fidelity, specificity and yield of a PCR run. In this study, variable concentration of $MgCl_2$ have been used to determine its effects on a PCR performance and yield. The template DNA, reaction buffer, dNTPs, primers and DNA polymerase were not changed throughout the study. The PCR amplicons were then electrophoresed, stained and then photographed using the UV light. Results showed that at 0.5 mM $MgCl_2$ concentration, no DNA band was produced in the gel. However, with an increased or excess in $MgCl_2$ concentration (3.0 mM to 6.0 mM) multiple bands of non-specific products were formed and band intensity decreased. The optimal $MgCl_2$ concentration was found to be 1.5 mM. At this optimized PCR amplification, the DNA band appeared as a single, bright band. For this and other reasons, it is deem necessary to optimize specific PCR amplification with respect to this divalent cation.

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INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Polymerase Chain Reaction (PCR) is an *in vitro* technique to synthesize large quantities of a given deoxyribonucleic acid (DNA) molecule (Saiki, 1989). PCR separates the DNA into their two complementary strands, synthesizes new template molecules using DNA polymerase and repeats this process very quickly. At the end of PCR cycle a logarithmic amplification of short DNA sequences (100 to 600 bp) within a longer double stranded DNA molecule will be produced. Polymerase chain reaction (PCR) was invented in the early 1980s by an American Scientist Kary B.Mulis at the Cetus Corporation (Mullis and Faloona, 1987).

The PCR steps are all carried out, one after the other, in bouts of cycling process. Specific PCR product are synthesized by a repetitive cycles of DNA denaturation step (about 1 min at 95°C), primer-template step (about 1 min at temperatures ranging between 45°C and 60°C) and primer extension step (for 1 min at 72°C) (Erlich, 1989). Since its discovery, PCR has been applied in many research areas such as molecular biology, genetics, forensics, pharmacogenetics, disease diagnosis and new application are still being explored.