UNIVERSITI TEKNOLOGI MARA (UiTM)

INVESTIGATION OF THE FACTORS THAT INFLUENCE LONG RANGE PCR

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ABSTRACT

Long range PCR method for uniform amplification of specific lambda DNA sequence is described. The careful design of all 6 pairs of primers and optimum temperature cycling profile produce good result for long range PCR. Three pairs of 15kb and three pairs of 20kb primers were design using oligo explorer 1.2. Four out of six (All three 15kb PCR and one 20kb PCR) produce results. Two variables that affect the efficiencies of long range PCR were studied which consists of the percentage of DMSO added and the concentration of $MgCl_2$ used in the PCR. A combination of 5%, 7%, and 10% DMSO were added to study the improvement of the efficiencies of long range PCR amplification that theoretically should increase the ratio of full-length products to shortened products. Results were somehow unobtainable and optimum percentage of DMSO for long range PCR is not known. The differing $MgCl_2$ concentrations were added and the result was also unobtainable and optimum concentration of free Mg^{2+} in the PCR mix is not known.

CHAPTER 1

INTRODUCTION

1.1 LONG RANGE PCR

Long range PCR allows the amplification of PCR products, which are much larger than those achieved with conventional Taq polymerases. Long-distance PCR would also make it possible to isolate large genomic fragments from complex genomes, as well as from hybrid cell lines or from microdissected or flow-sorted chromosomal regions. (Nelson *et al.*, 1989)

The ability to amplify fragments up to 20-50 kb would potentially enable the isolation of an entire gene from a cDNA, thereby obviating the time-consuming task of screening a genomic library for the target gene. For example, the generation of a 20-kb fragment would span approximately half of the DNA cloned into a cosmid, thus making it possible to access the entire insert from as few as two amplifications initiating from the left and right vector cloning sites.