UNIVERSITY TEKNOLOGI MARA

AMPLIFICATION OF WHOLE mRNA IN A549 ADENOCARCINOMA CELL BY TEMPLATE-SWITCHING METHOD

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CHAPTER 1

1.0 INTRODUCTION

1.1 Research Background

Polymerase Chain Reaction (PCR) is a DNA amplifying technique pioneered by Kary Mullis at Cetus Corporation in the early 1980's (Handyside et al., 1989)

PCR is a technique amplifying a specific DNA component from very small quantities of DNA source component and also from the source with poor quality into numerous of copies.

It involve series of steps that is denaturation, annealing and primer extension. For the first steps is denaturation process, the DNA is heated to 95°C to unwind the helical structure into single stranded DNA. Next step is the annealing process where complementary strand are hybridized with primers. The temperature used during this step depended on the primer size and its homology to the target DNA.

Last step is primer extension, this step where extension occur catalyzed by thermostbale DNA. Taq polymerase is used for extension step ("Polymerase Chain Reaction (or PCR)," 2006).

1.2 Statement of problems

Upstream analysis of total RNA from biopsy samples are often hampered by the limited amount of samples obtained. This hindered detailed analysis of the transcriptome which could potentially shed new light in gene expression profile in both healthy and disease state. A reliable method to linearly amplify the expressed messenger RNAs (mRNAs) from a limited amount of total RNA will be of great help for archiving purpose and also for upstream analysis.

1.3 Objective

To culture A549 lung adenocarcinoma cell line / lung small airway epithelial cell line.

1. To isolate total RNA from the cultured cell line.
2. To convert the whole population of mRNA in the total RNA into 1^{st}-strand cDNA by using reverse transcription template-switching method.
3. To linearly amplify the 1^{st}-strand cDNAs using long-range PCR