UNIVERSITI TEKNOLOGI MARA

AMPLIFICATION OF miRNA 19B AND 92A (MCF-7 CELL LINE) FROM PGEM®-T EASY CLONING VECTOR

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Abstract

miRNAs are non-coding RNAs that can regulate the gene expression in numerous biological processes in our body and these include miR-19b and miR-92a that involved in the expression of MCF-7 breast cancer. These miRNAs had been cloned in pGEM® -T Easy Cloning Vector and stored for 2 years. The aims of this study were to design specific primer for PCR, amplify the gene and verify the region of interest by agarose gel electrophoresis and DNA sequencing before retrieving it for further study. There were possibilities that the gene had been mislabeled, or might have undergone mutation while in the storage. The result of agarose gel electrophoresis demonstrated the size of the amplicons was approximately 500 bp when compared to DNA ladder, reflecting the expected band size of miR-19b and miR-92a of MCF-7 which was 488 bp. The analysis of chromatogram from DNA sequencing indicated sharp and clean peaks with no background noise. Besides, the sequence alignment showed a complete match of 100% similarity with the sequence *Homo sapiens* miR 17-92 cluster from NCBI database. The results from this study suggested that the cloned gene was still in a good condition even after 2 years. It was verified that there was no change in the DNA sequence or mutation in the region of interest. Verification of the desired region is necessary prior performing a further study as it allows researchers to detect the errors from the beginning hence saving resources, time and energy.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

MicroRNA (miRNA) is a small, single stranded RNA molecule which does not encode proteins. This short RNA about 20 nucleotides in length can regulate the expression of gene post-transcriptionally by binding complementarily to 3' or 5' untranslated region (UTR) of messenger RNA (mRNA). The consequence of the binding can result in inhibition of protein translation or degradation of mRNA (Almeida, Reis, & Calin, 2011).

Bioinformatics data evaluates about one third of all human genes are regulated by miRNAs (Tétreault & De Guire, 2013). These tiny RNAs regulate the gene expression in numerous biological processes in our body such as cell proliferation, differentiation, apoptosis as well as metastasis (Almeida *et al.*, 2011). Alteration in the miRNA expression could lead to many diseases including cancer (Sassen, Miska, & Caldas, 2008).

Before the discovery of miRNAs, most studies are focused on coding RNAs and the proteins which they encode. The classical central dogma of molecular biology has put forth the studies on non-coding RNAs. Scientists assure that DNAs have to be transcribed into mRNAs and later undergo translation process for making proteins which play important roles in human body and could be manipulated for new drug