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# AMPLIFICATION OF miRNA 19 AND 20 (HEP G2 CELL LINE) FROM pGEM®-T EASY CLONING VECTOR

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# ABSTRACT

Hep G2 is a cell line derived from a liver tissue with hepatocellular carcinoma. MicroRNAs (miRNAs) which act as post-transcriptional regulators of gene expression in diverse cellular and developmental processes contribute to the progression of many types of cancers. Over expression of miRNA-19 and miRNA-20 has been found in hepatocellular carcinoma and considered as an oncogene (Mogilyansky and Rigoutsos 2013). This research was performed in order to amplify and verify miRNA 19 and 20 derived from Hep G2 cell line that has been cloned in PGEM®-T Easy Cloning Vector via Polymerase Chain Reaction. The verification of the amplicons was performed via gel electrophoresis, DNA sequencing and sequence alignment. The result obtained from the gel electrophoresis indicated that the size of the DNA sequence miR-19 and miR-20 was slightly larger than 500 bp. DNA sequencing showed that the analysed sequenced from the chromatogram was clean with evenly spaced peak and without any background peak or noise observed. The sequence miR-19 and miR-20 from Hep G2 cell line that was aligned with the sequence obtained from the NCBI, showed 99.6% similarities due to the presence of a point mutation of the cloned DNA sequence inside pGEM®-T Easy Vector. The BLAST results showed that the amplicons being verified originated from *Homo sapiens* miR-17-92 microRNA cluster containing miR-19 and miR-20 and it can be stipulated that the cloned miR-19 and miR-20 inside the pGEM®- T Easy Vector was still reliable despite the presence of a single point mutation on the DNA sequence.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

In the world of biotechnology, microRNA (miRNA) is considered as a new knowledge. It was being recently discovered in 1993 and since then, the long known theory of gene regulations has been redefined (Bhaskaran and Mohan 2013). Victor Ambros and colleagues found out that a gene known to control the timing of larvae development for *C. elegans,* does not code for a protein but it is actually produces a pair of small RNAs of approximately 22 and 61 nucleotides in length (Tetreault and De Guire 2013) and these small RNAs are identified as microRNAs. MicroRNA is introduced as an endogenous small noncoding RNAs that regulate gene expression not only in animals but also in plants (Ul Hussain 2012). These microRNAs function as post-transcriptional regulators usually by base pairing to the mRNA to either repress or promoting protein synthesis.

Since the discovery of nearly twenty three years ago, it has shown that miRNAs have a significant role in modulating various processes by taking part in the normal physiological homeostasis as well as portraying its role in the pathogenesis of major diseases such as cancer. Other than that, miRNAs also contribute to a diverse cellular process including cell proliferation, apoptosis also in the progression of a disease