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AMPLIFICATION OF miRNA 19B AND 92A (HEP G2 CELL LINE) FROM PGEM®-T EASY CLONING VECTOR

SITI HAJAR BINTI JAMALUDIN

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ABSTRACT

There are six miRNAs consisted in miR-17-92 cluster where two of the known types of the microRNAs expressed by Hep G2 (human liver cancer line) (Molin Li et al., 2014). miR-19b is known to act by down-regulating the phosphatase and tensin homolog (PTEN) which act as tumour suppressor gene in liver cancer (Budhu et al., 2008) while miR-92a family induce tumorigenesis (Molin Li et al., 2014). This research was undergone to amplify those miRNAs that had been cloned into the pGEM®-T Easy cloning vector to verify its purity before being used in further cancer research. A specific primer was designed before PCR was initiated. However, instead of using the designed primer, a universal primer was used, which were M13 forward and reverse primers. The universal primer was more compatible and economically efficient to be used as pGEM®-T Easy cloning vector has the specific binding site for the M13 primers. After PCR was successfully done, the amplicons were analysed by automated sequencing and gel electrophoresis methods. From the gel electrophoresis, the size of amplicons were estimated to be lower than 500 bp, by referring to the DNA ladder, thus reflecting and correlate the size of amplicon to the actual size of the insert which is 488 bp. The sequence was then analysed via FinchTV where the generated chromatogram showed clean and well-resolved peaks together with the location of the sequences of miR-19b and miR-92a, indicating the region amplified did contain the interest sequence. The sequence later was aligned with the reference sequence retrieved from NCBI by using online tools Clustal Omega and BoxShade. The sequences were perfectly aligned, indicating no mutations had occur.

CHAPTER 1

INTRODUCTION

1.1 Study Background

MicroRNAs (miRNAs) are small, non-coding and endogenous RNAs which have the capability in regulating gene expressions (Babashah & Soleimani, 2011). They are single stranded with length of around 22 nucleotides (Molin Li *et al.*, 2014, p. 92). As a type of noncoding RNA, miRNAs do not translated into proteins, instead, miRNAs are known as "RNA genes controlling other protein-controlling genes" (Appasani, 2008). Many researchers have extensively reviewed the biogenesis of miRNA (Zhang, Pan, Cobb, & Anderson, 2007). The general overview of the miRNA biogenesis starts with RNA polymerase II which transcribe miRNAs as long primary RNAs (pri-miRNAs) before being processed into short hairpin structures by RNase III enzyme Drosha. Those short hairpin structures are known as precursor miRNAs (premiRNAs). Pre-miRNAs consequently processed into mature miRNAs by RNase III enzyme Dicer (Min Li *et al.*, 2009).

MicroRNAs were firstly discovered in *Caenorhabditis elegans* by Nobel Prize winners, Dr. Andrew Fire, and Dr. Craig Mellow (Min Li *et al.*, 2009). They found