

UNIVERSITI TEKNOLOGI MARA

**CLONING OF miRNA-19A AND miRNA-20A FROM
HEPG2 LIVER CANCER CELL LINE**

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ABSTRACT

MicroRNAs (miRNAs) are class of small, endogenous short non-coding molecules that involved in the regulation of gene expression by degradation or repressing translation of mRNA to protein by targeting at the 3'UTR. A growing body of studies suggested that miRNAs were highly expressed in human to cause the development of cancerous cell including liver cancer cell (HepG2). The aim of this study is to clone the miRNAs 19a and miRNA 20a from HepG2 and MCF-7 by using the specific primers that have been designed from the NCBI database. From the beginning, PCR was done to amplify the region of miRNA19a-20a from HepG2, MCF-7 and WRL. The optimum temperature (63.1°C) that was used in both PCR was the same and a clear band can be seen under the UV light. The PCR product was inserted in pGEMT Easy cloning vector by using TA cloning approach. The succesful of cloning can be screened through blue-white screening where the appearance of white colony can only be seen in the agar plate that contained miRNA genes from HepG2. The white colony was propagated overnight. Plasmid identification was done to ensure the white colony contained the plasmid with the desired insert. To prove that there was an insert inside the vector, PCR by using M13 primers and sequencing analysis was carried out. The size of the band for the PCR product was expected, approximately 544bp. Through the sequencing analysis, it was found that one of the base has undergone point mutation. However, the sequencing analysis must be carried out in triplicate to validate this point mutation.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Cancer is defined as a disease in which abnormal cells divide without control and are able to invade other tissues (Clark, 1991). Even, cancer cells can spread to other parts through the blood and lymph systems (Clark, 1991). Until 31 December 2007, it has been statistically demonstrated by Ministry of Health that cancer is the third cause of death in Malaysia (Appendix 1.1). This number can grow higher if it is not being controlled.

Many studies have been carried out to understand the nature of cancer, its effects and ways to overcome it (Gadducci, Cosio, & Genazzani, 2006). The major treatment including surgery, radiotherapy and chemotherapy drug need to be carefully select in order to ensure the cancer can be removed from the body (Amagase, Arakawa, & Hashimoto, 1989). Surgery or radiation therapy alone is