UNIVERSITI TEKNOLOGI MARA

CLONING OF MiRNA 19b AND MiRNA 92a GENES FROM HEPG2 CANCER CELL LINES AND MCF-7 CANCER CELL LINES

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ABSTRACT

MicroRNAs (miRNAs or miRs) are small RNA molecules of approximately 22 to 28 nucleotides which a class of endogenous encoded non-coding RNAs that control gene expression. Previous researchers suggested that miRNAs were highly expressed in human resulting to the development of various cancerous cells including the liver cancer cell (HepG2) and breast cancer cell (MCF-7). This study aims to clone the miRNA 19b and miRNA 92a from HepG2 and MCF-7 cancer cell lines by using a set of specific primer designed with NCBI Genebank. First and foremost, the miRNA 19b and miRNA 92a from HepG2 and MCF-7 cancer cell lines as well as WRL healthy cell line was amplified using polymerase chain reaction (PCR). However, WRL cell line was discontinued in the bulk PCR. The optimum annealing temperature used was 63.1°C after several optimization processes was done. Clear band was observed under the UV light. PCR product was further cloned using TA cloning method. Successful cloning was screened through blue white screening procedure. White colonies were observed in agar plates. This indicated the successful ligation of the vector and inserts which is miRNAs from HepG2 and MCF-7. The resulting white colony was propagated 16 hours. Plasmid identification was done to ensure white colony contain the plasmid and desired insert. In order to prove that there was an insert in the vector, PCR by using M13 primers and sequence analysis was carried out. The size of PCR product was expected approximately 488bp. Through sequence analysis, both sequences for HepG2 and MCF-7 were aligned according to NCBI Genebank sequences.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

MicroRNAs (miRNAs or miRs) are small RNA molecules of approximately 22 to 28 nucleotides which a class of endogenous encoded non-coding RNAs that control gene expression. It acts by targeting sequence for degradation and/or transitional repression (Cullen, 2004). Since the discovery of small non-coding RNAs lin-4 and let-7 (currently known as miRNAs) in *Caenorhabditiselegans*, hundreds of miRNAs sequences have been so far identified in a wide range of organisms from plants to humans (Cai, Yu, Hu, & Yu, 2009).

MiRNAs play important roles in regulating a variety of biological process such as proliferation, differentiation and apoptosis. It has been demonstrated that miRNAs have a crucial function in oncogenesis by regulating cell proliferation and apoptosis as oncogenes or tumour suppressor as well as maintenance of stemness thus their dysregulation (miRNAs) contributes to many human disease