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AMPLIFICATION OF THE PURINE-RICH REGION FROM UPSTREAM OF MIRNA17-92A SEQUENCE FOR TRIPLE-HELIX STUDY

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ABSTRACT

In the human body, there are the existence of sequences that are specific for the formation of triple helix. The triplex-forming oligonucleotides (TFO) might be very useful therapeutically for example in suppressing cancer. This review generally describes the process of generating the template for the TFO binding site. A very short sequence of template which is 18bp from the hepG2 cancer cell line were amplified using the specially designed primers by polymerase-chain reaction (PCR). Amplifying such short sequence may encounter problem like the formation of secondary structures such as primer dimer and hairpin loop. The desired region of PCR product was purified and sent for direct sequencing to verify the sequence. This study encourages further steps and processes that need to be done to study on TFO.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Under normal condition DNA exists in a duplex form. However, under certain circumstances, DNA takes a triplex structure which is either intramolecular or intermolecular. The triplex is sequence-specific and formed by the addition of the third strand to the major groove of the duplex. Triplex-forming-oligonucleotides (TFOs) have numerous functions such as site-directed mutagens, repressors of transcription, and inhibitors of replication. TFOs specifically recognize the homopolypurine:homopolypyrimidine sequences present in eukaryotic genes. TFOs can be formed by two ways. First is the polypyrimidine strand binds parallel to the polypurine strand of DNA by Hoogsteen hydrogen bonds. Secondly is in which the polypurine third strand that is commonly G-rich binds antiparallel through reverse-Hoogsteen hydrogen bonds. The parallel structures are stabilized in acidic condition whereas antiparallel structures are generally independent of pH (Guntaka, Varma, & Weber, 2003).

In the past few decades, viral vectors have been extensively studied for the use in gene therapy. DNA strategies are developed to correct specific mutations. TFOs technology is based on the potential of binding of the single-stranded oligonucleotides