UNIVERSITI TEKNOLOGI MARA

GENERATION OF GENE KNOCKOUT CASSETTE (GKO-Ab^{R}) FOR CLAUDIN-1 GENE TARGETING

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ABSTRACT

Hepatitis C is a type of inflammation of the liver, origin of Hepatitis C Virus (HCV) and it is estimated that more than 170 million people worldwide have been infected with this virus. Claudin-1 (CLDN-1) is one of the receptors that are believed to be crucial for HCV entry process, but the details on the mechanisms are still unclear. This research aims to design the primers and amplify the antibiotic resistance gene of interest from the commercially available plasmid and to produce gene knockout cassette of claudin-1 by linking the homology arms of exon 1 claudin-1 region. PrimerBlast online software was used as the tool to design the target-specific primers of pcDNA3.1 and pCMV/Bsd vector. The gene of interest used from the pcDNA3.1 vector was Neomycin resistance gene while from pCMV/Bsd vector the gene of interest used was Blasticidin resistance gene. The sizes of the primers produced for pcDNA3.1 and pCMV/Bsd generate the antibiotic resistance gene cassette by using Polymerase Chain Reaction (PCR) amplification together with 0.2µl Phusion DNA polymerase (1U/50µl). All PCR products were purified and analyzed with 2.0 % agarose gel electrophoresis method by running alongside with 100bp DNA Ladder Marker and resulting in successful generation of the gene knockout antibiotic resistance gene (GKO-Ab^R) cassette. It is estimated that these cassettes could be used for further study which are to bind with the CLDN-1 homology arms (left and right), to construct the CLDN-1 knockout plasmids and to be used in gene knockout targeting work for CLDN-1 in HCV permissive cells.

Keywords: Hepatitis C virus, Claudin-1, gene knockout, homology arms vector were estimated to be around 1.5 kb and 1.7 kb respectively. Then, the primers were used to

CHAPTER 1

1.0 INTRODUCTION

1.1 Introduction

Gene targeting technology has been widely used in studying gene function in human cells. It can be used to treat diseases, especially involved in gene problems, such as Down syndrome and this technique also can be applied to culture human cells (Bunz, 2002). Homologous recombination can be applied in various types of cell for gene disruption, including in somatic human cell (Kohli, Rago, Lengauer, Kinzler, & Vogelstein, 2004).

Based on previous studies, in order to inactivate genes in cultured cells, several methods have been developed. One of the methods is RNA interference (RNAi) techniques, which was developed to knockdown gene expression. This technique is more rapid and adaptable to high-throughput approaches, compare to gene targeting techniques (Rago, Vogelstein, & Bunz, 2007). However, results from RNAi method are difficult to interpret and analyze because of non-specific effects, and varied between laboratories and experiments (Kohli *et. al.*, 2004). Therefore, a knockout technique is used in this study to improve the interpretation of the results.