UNIVERSITI TEKNOLOGI MARA

GENERATION OF NEOMYCIN RESISTANCE GENE KNOCKOUT PLASMID (pGKO – Neo^R) FOR SCAVENGER RECEPTOR CLASS B TYPE 1 (SR-B1) GENE

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ABSTRACT

Hepatitis C is a contagious liver infection that caused by Hepatitis C virus (HCV). It can range in severity from a mild illness lasting for a few weeks to a serious and lifelong illness. HCV infection is endemic worldwide and about 3% estimation by WHO on the prevalence of HCV infection representing from 123 million people. SR-B1 has been reported to have a significant role in HCV attachment and entry into human hepatocytes but the mechanism of entry is not very fully understood. The aim of this research is to produce Neomycin resistance gene knockout plasmid (pGKO-Neo^R) by fusion of right and left homology arms of SR-B1 with the gene knockout antibiotic resistance gene (GKO-Neo^R). This will help in studying the relationship between SR-B1 in the attachment and entry mechanism. Other than that is to look and optimize the fusion PCR on production of HCV gene knockout. Human genomic DNA was used as DNA template to generate the left and right homology arms of SR-B1 for amplification of the gene. Neomycin resistance gene was the antibiotic resistance genes used in generation of SR-B1 gene knockout cassette. This step was done by previous student and I proceeded with the reamplification of the PCR product. Fusion PCR product done with left and right homology arm for SR-B1 fused to an Neomycin antibiotic resistance gene cassette via linker sequence. Gradient fusion PCR reaction was proceeded to optimize the fusion PCR reaction. As suggested by the protocol, 65°C is the best annealing temperature for fusion PCR reaction. Based on the result there was possibly a fuse band of either LHA + Neo^R or Neo^R +RHA because according to the DNA ladder the band appeared at size around 2kbp to 3kbp even though the band was not clear enough. The targeted size of full fusion PCR product is 3.7kbp but if the size is about 2.7kbp it might show that, it is a partial fusion PCR product. There are also multiple bands appeared which could come from impurites of the sample from previous PCR product and might be the crucial factor in order to obtain a clear and fine band yield on the agarose gel. I am suggesting that the PCR products be purified using gel extraction kit before proceeding to the next reaction. Hopefully, the production of pGKO – Neo^R could assists and supports other research studies on HCV entry mechanism.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Hepatitis is an inflammation of the liver which is usually caused by a viral infection. There are several types of hepatitis, referred as types A, B, C, D and E (WHO, 2013). Hepatitis C virus (HCV) was recognised as a main cause of chronic liver disease since its discovery in 1989. This virus mostly spread due to exposure to infective blood (Shepard, Finelli, & Alter, 2005). Based on the potential circumstances of susceptivity, the disease onset is rarely recognized by infected persons who develop acute hepatitis C (Seeff, 2002).

The hepatitis C virus (HCV) is a blood-borne pathogen that emerges to be endemic in most areas of the world. Variation in virus and host specific factors are likely responsible for the individual differences observed in the natural history of HCV related chronic disease and in response to antiviral therapy. Differences in the frequency and extent to which various risk factors contribute to the transmission of HCV are responsible for temporal and geographic differences that have been observed in the epidemiology of hepatitis C (Alter, Hutin, & Armstrong, 2000). This infection may occur via transfusions of HCV contaminated blood and blood products, through injection of drug using contaminated injections equipment and during