UNIVERSITI TEKNOLOGI MARA

AMPLIFICATION OF PURINE RICH REGION FROM COL4A3 FOR TRIPLE HELIX STUDY IN KERATOCONUS EYE DISEASE

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ABSTRACT

Keratoconus (KC) eye disease is a non-inflammatory disorder characterized by eye bulging due to corneal thinning and results in blurred vision and astigmatism. Several factors lead to KC development include genetic factor and polymorphism of COL4A3 might cause KC through decrease in collagen production. Application of triplex forming oligonucleotide (TFO) based drugs may be hopeful method to treat KC. Triple helix formed when third single strand of DNA fragment bind to DNA duplex in antiparallel form to purine rich region by reverse Hoogsteen hydrogen bonds and able to suppress gene expression by inhibits the initiation of transcription or through recombination and mutagenesis that cause permanent changes to DNA. From NCBI (NG_011591.1) database, 32bp of purine rich region was identified and appropriate pair of primer was designed using Oligo Software. The amplicon then subjected for gel electrophoresis and desired product size was successfully obtained. The amplicon was then sent for direct sequencing to determine the sequence of the potential target site for KC treatment. Based on the sequencing analysis, 88.8% of amplicon aligned with original sequence from NCBI and purine rich region was identified from the sequence. This shows that the purine rich region of COL4A3 gene was successfully amplified and can be used by other researcher for further study.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Keratoconus (KC) eye disease is a non-inflammatory disorder characterized by eye bulging basically due to progressive corneal thinning (Bykhovskaya et al., 2016) and results in blurred vision, myopia, astigmatism and increased sensitivity to light (National Eye Institute (NEI), 2016).

The prevalence and incidence of KC are variable between region, ethnicity and gender. As reported, the prevalence of Russian was 1 in 500 000 population (0.0003%) (Gorskova et al., 1998). While for Asia region, central India was 2.3% among Indians aged 30 years and above (Jonas et al., 2009), Denmark is 86 per 100 000 population (0.086%), Finland is 30 per 100 000 population (0.03%) and in Japan is 17.3 per 100 000 population (0.0173%) (Kok, Tan, & Loon, 2012).

In Malaysia, study conducted by Mohd-Ali and coworkers reported that the prevalence recorded was 1.2% with roughly 1 per 100 populations (Mohd-Ali et al., 2012). However, this is not an indicator for KC frequency in Malaysia since some