

UNIVERSITI TEKNOLOGI MARA

**AMPLIFICATION OF PURINE RICH REGION
FROM DOCK9 GENE FOR TRIPLE HELIX
STUDY IN KERATOCONUS EYE DISEASE**

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ABSTRACT

Triple helix are formed by binding a scientific triple helix forming oligonucleotides (TFO) with high affinity and specificity to purine rich region in the major groove of homopurine sequence in the double stranded DNA (Chan & Glazer, 1997). In vitro study shows that triple helix formation alter the double helical conformation of DNA and inhibit process transcription and replication in DNA. The aim of the study is to amplify the purine rich region in DOCK9 gene for triple helix study in Keratoconus (KC) eye disease. DOCK9 gene were discovered as a candidate gene for KC which responsible in cytokinesis of the cell (Bykhovskaya, Margines, & Rabinowitz, 2016). At first, the purine rich region was amplified using a pair of primer designed based on the sequence of DOCK9 gene region obtained from NCBI. PCR cycle then performed with an optimum annealing temperature at 51°C. Expected sizes of PCR products were successfully obtained through agarose gel electrophoresis. Direct sequencing were done to verify the sequence of this potential TFO target site. Through direct sequencing results and alignment, purine rich sites were successfully amplified and verified. These potential TFO binding sites might be useful for specific inhibition in gene expression.

CHAPTER ONE

INTRODUCTION

1.1 Background Of Study

Prevalence and incidence of Keratoconus (KC) are variable in different region of the world. There are so many factors that may contribute to this variability include environmental, geographic location, ethnicity, and group of patient included in the study. In Asir Province, Saudi Arabia, the incidence of KC reported is 20 cases per 100 000 population (Assiri, Yousuf, Quantock, & Murphy, 2005). In Olmsted County, Minnesota, studies found that incidence rate was 2 per 100 000 population and prevalence rate was 54.5 per 100 000 population from the year 1935 to 1982 (Kennedy, Bourne, & Dyer, 1986). The Central India Eye and Medical Study state that among Indians aged 30 years and above and live in a rural area of Central India, KC has a prevalence of $2.3\% \pm 0.2\%$ (2300 per 100 000) (Jonas, Nangia, Matin, Kulkarni, & Bhojwani, 2009).

The Asians incidence of KC is very high as 25 per 100 000 (1 in 4000) per year is affected with this disease (Georgiou, Funnell, Cassels-Brown, & O'Connor, 2004). Caucasians show a much lower incidence which is 3.3 per 100 000 per year (Georgiou et al., 2004). Asians are more likely to develop KC than Caucasian (Georgiou et al., 2004). In comparison with white patients, Asians have fourfold increase in incidence, younger and require corneal grafting at an earlier age