UNIVERSITI TEKNOLOGI MARA

# AMPLIFICATION OF PURINE RICH REGION FROM *VSX1* GENE FOR TRIPLE HELIX STUDY IN KERATOCONUS EYE DISEASE

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# TABLE OF CONTENTS

# ABSTRACT

Keratoconus is a disease affecting the cornea of the eyes, which could impaired the vision of patient. The application of triple helix study for inhibiting the transcription of the DNA in *VSX1* gene is vital pertaining to suppress this disease as it may open promising strategies for an accurate treatment. The concept of triple helix study is the binding of poly-purine DNA strand to major groove of double helix double strand via reverse-Hoogsteen base pairing. Hence, triple forming oligonucleotide (TFO) will inhibit transcription, the process where DNA being transcribed into RNA and prevent replication. This study generally described the process of recognizing purine rich site region in the *VSX1* gene of cornea cell. From NCBI database, 22 bp purine rich region were identified in the *VSX1* gene, making it specific site for the binding with the TFO to inhibit the transcription. PCR primers were designed using NCBI Primer Desinging Tools and the expected size of PCR product (321bp) was successfully obtained. Agarose gel electrophoresis analysis and direct sequencing using forward and reverse primers were carried out to ensure the sequences have potential for TFO target sites. Sequencing results apparently shown that purine rich site were successfully amplified and verified.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of study

Keratoconus is a bilateral, non-inflammatory progressive corneal disease, which arrests people basically in late teenage years and sustain for 10 to 20 years upon arrival. Cases of unilateral keratoconus are rare but can be seen occasionally in clinical practice. It almost affects both of the eyes but act particularly in each eye. For example, the first eye develop the disease has a more marked progression instead of the other eye. There is no difference in prevalence between males and females and it affects 1 in 2000 people (Yahalom et al, 2005). It causes cornea to be conical in shape, via the progression of thinning the cornea, as it originally be in dome-shaped (Al-Raddadi et al., 2016). Besides, the development of keratoconus relates with physiology of the cornea in the terms of its function in serving as protective barrier and refracting the light entering the eye. Since cornea is part of the eye that refracts about 60% of light, altering of its shape will distort function through inhibition of light from entering and focusing accurately into the eye. The condition may be worse if there is scarring of tissue and swelling, which may impair the vision of eye. More advanced signs of keratoconus such as Fleischer ring and Munson's sign follow as the disease progresses (Tomalla & Cagnolati, 2007).