



Faculty of Resource Science and Technology

**Mutation Analysis of the Human Ribosomal Protein S8 (*uS8*) Gene in
Nasopharyngeal Carcinoma (NPC) Cell**

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**Bachelor of Science with Honours
(Resource Biotechnology)
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**Mutation Analysis of the Human Ribosomal Protein S8 (uS8) Gene in
Nasopharyngeal Carcinoma (NPC) Cell**

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A Thesis Submitted in Partial Fulfilment of the Requirement for The Final Year Project 2
(STF3015) Course

Supervisor: Associate Professor Dr. Edmund Sim Ui Hang

Resource Biotechnology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

2018

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ABSTRACT

Nasopharyngeal carcinoma (NPC) is one of the major health problem in Malaysia, reported to be the fourth most common cancer among Malaysians and the third most common cancer among Malaysian men. The mechanisms which cause the disease is not fully understood because the understanding about NPC-associated gene is unclear and how ribosomal protein (RP) genes become part of it. In this study, human ribosomal protein S8 (*uS8*) gene is studied to find out whether or not the mutation of this gene is the cause of differential expression of the ribosomal protein gene which lead to NPC. The objective of this project is to isolate the sequence of *uS8* gene in HK1 cell lines and to analyse the sequence of *uS8* gene isolated from the HK1 cell lines. In this study, a set of primer is designed by using NCBI primer blast, Oligo Calc, and OligoAnalyzer 3.1. Then, gradient PCR is used to amplify the DNA of interest. However, both objectives are not achieved at the end of this project. There is no amplification of target gene from the gradient PCR as no band is observed from the gel visualization. To conclude, technical error must be reduced and to study about mutation analysis, biological replicates should be conducted instead of studying on one type of cell line only.

Keywords: Mutation analysis, Nasopharyngeal carcinoma (NPC), *uS8* gene, HK1, Ribosomal protein gene

ABSTRAK

*Kanser Nasofarinks (NPC) adalah salah satu masalah kesihatan utama di Malaysia, dilaporkan menjadi kanser yang ke-empat paling banyak di kalangan rakyat Malaysia dan kanser ketiga paling umum di kalangan lelaki di Malaysia. Mekanisma yang menyebabkan kanser ini belum difahami sepenuhnya kerana pemahaman tentang gen yang berkaitan dengan NPC tidak jelas dan bagaimana gen protein ribosom (RP) menjadi sebahagian daripadanya. Dalam kajian ini, gen manusia ribosom protein S8 (*uS8*) dipelajari untuk mengetahui sama ada mutasi gen ini adalah penyebab perbezaan gen protein ribosom yang membawa kepada NPC. Objektif projek ini adalah isolasi gen *uS8* dalam sel HK1 dan untuk menganalisis gen *uS8* yang telah diisolasikan daripada sel HK1. Dalam kajian ini, primer direka dengan menggunakan primer-blast NCBI, Oligo Calc, dan Oligo Analyzer 3.1. Kemudian, PCR gradien digunakan untuk mengamplifikasikan DNA yang disasarkan. Namun, kedua-dua objektif tidak tercapai pada akhir projek ini. Ini kerana tiada penambahan gen sasaran dari PCR di mana tidak ada fragmen DNA yang dilihat dari visualisasi gel. Kesimpulannya, kesilapan teknikal mesti dikurangkan dan untuk mengkaji tentang analisis mutasi, replikasi biologi perlu dijalankan dan bukannya mengkaji satu jenis sel sahaja.*

Kata-kata kunci: Analisis mutasi, Kanser nasofarinks, gen *uS8*, HK1, Gen ribosom protein

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LIST OF ABBREVIATIONS

bp	base pair
cDNA	complementary DNA
DNA	deoxyribonucleic acid
DNTPs	deoxynucleosides triphosphate
EBV	Epstein-Barr Virus
EDTA	ethylenediamine tetra-acetic acid
EtBr	ethidium bromide
g	gram
IARC	International Agency for Research Cancer
kb	kilobase
M	Molar
mRNA	messenger RNA
M-MLV	Moloney Murine Leukemia Virus
NCBI	National Center for Biological Information
NCR	National Cancer Registry
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RPG	Ribosomal Protein Gene
rpm	rotation per minute

RT-PCR	Reverse Transcription-PCR
TAE	tris-acetate EDTA
TE	tris EDTA
Tris	tris (hydroxymethyl) aminomethane
<i>uS8</i>	human ribosomal protein S8 gene
UV	ultra violet
μ l	microliter
V	volts

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CHAPTER 1: INTRODUCTION

Ribosome is the cellular organelle which have two equal subunits, 40S as a small subunit and 60S as a large subunit and this include few specific RNA molecules and larger proteins which are different in shape, size and topology (Wool, 1979). Ribosome plays a major role in protein synthesis. During evolution, the ribosomal proteins (RPs) are conserved which means that the it has higher percentage of identical amino acid sequences between mammals. Therefore, RPs are very important in organisms even though they have different specificity functions in a mature ribosome (Ruggero & Pandolfi, 2003).

Problems with regard to the translational process happens when there is a genetic defect in ribosomal components or ribosomal protein genes (RPGs) and thus most researches assume RPs only involve in protein synthesis. However, most RPs are also related to various roles of protein biosynthesis called extra-ribosomal fun4ctions (Vaarala *et al.*, 1998). The downregulation of cell growth and apoptosis is functional and morphological changes of tissues which lead to cancer (Ruggero & Pandolfi, 2003). For example, there is downregulation of three 40S RPGs and a 60S RPGs in nasopharyngeal carcinoma (NPC) cell lines (Sim *et al.*, 2017).

RPGs mutation or disturbance in the level of expression are found in many inherited genetic diseases such as Diamond-Blackfan anaemia syndrome, Noonan syndrome and Tuner syndrome (Yang & Liu, 2005). The disease resulting from the abnormal expression of RPGs is supported by the fact that RPGs are well-known as a housekeeping gene that should be constantly expressed for cellular regulation (Eisenberg & Levanon, 2003).

Nasopharyngeal carcinoma is a cancer originating mainly from the nasopharynx epithelium (refer to Figure 2.2 at the Literature Review section). It is one of the types of cancer occur in the head and neck region, frequently occur at the opening of Eustachian tube in the Rosenmuller's fossa (Chan *et al.*, 2015). Nasopharyngeal carcinoma (NPC) is a major health problem in Malaysia, reported to be the fourth most common cancer among Malaysians and the third most common cancer among Malaysian men (Omar & Tamin, 2011). Genetic susceptibility in NPC is well-known, however, the mechanisms which cause the disease is not fully understood. This is because the understanding about NPC-associated gene is unclear and how ribosomal protein (RP) gene becomes part of it (Sim *et al.*, 2017).

Initially, two RPGs namely, *eS26* and *eS27* reported to be associated with NPC and these genes were downregulated in NPC tumours (Sim *et al.*, 2008). However, this hypothesis which states that *eS26* and *eS27* as NPC-associated gene was denied as another RPG, *eL32*, was also found not related with NPC tumorigenesis (Ma *et al.*, 2012). This brings to the thought that only certain RPGs are associated with NPC. In this study, mutation analysis is performed to find out whether or not the mutation of human ribosomal protein S8 (*uS8*) gene is one of the cause of differential expression of the ribosomal protein gene which lead to nasopharyngeal carcinoma and hence study the association of the *uS8* gene with NPC.

The objectives of this project are:

1. To isolate the sequence of *uS8* gene in HK1 cell lines
2. To analyse the sequence of *uS8* gene isolated from the HK1 cell lines

CHAPTER 2: LITERATURE REVIEW

2.1 Ribosomal Proteins

The ribosome is the cellular organelle that plays an important role for the protein synthesis in all cells, referred to as translation. Eukaryotic ribosomes have two subunits, 40S as a small subunit and 60S as a large subunit and this include few specific RNA molecules and larger proteins which are differ in shape, size and topology (Wool, 1979). Other than protein synthesis, ribosome is also important for regulation cell growth and death as these processes involve drastic changes in gene expression patterns and therefore, ribosome biogenesis is crucial as a functional translational apparatus (Naora & Naora, 1999).

Ribosomal proteins are vital in regulating cell growth and death as it is part of the integral components of a basal cellular machinery which act as a mediator of maintenance and change of cell state. However, there is evidence saying that certain individual ribosomal proteins are able to directly act as modulators and effectors of change of cell state (Naora & Naora, 1999).

2.1.1 Ribosomal Protein Genes (RPGs)

Ribosomal protein genes (RPGs) are important tools for studying intron evolution. RPGs are well-known for their constantly regulated expression and thus called a house-keeping gene. Through recent high-throughput studies, it provides an opportunity to study how human RPGs are modulated and how the transcriptional regulation might contribute to the gene expression coordination in various tissues and cell types (Li *et al.*, 2016). There are 80 RPGs in the human genome, in which all are in a single-copy gene (Rodnina & Wintermeyer, 2011). Under every condition in human cell type, these genes are active. The level of mRNA expression is highly correlated in different cell types, species and experimental conditions (Meyuhas & Perry, 1980).

2.2 Anatomy of Nasopharynx

The nasopharynx is located at the upper part of the throat located behind the nose. It is part of the pharynx which separated in three segments namely the nasopharynx, oropharynx and the hypopharynx. The nasopharynx is located at the space above the soft palate at the back of the nose (Figure 2.1a). The soft palate separates the nasopharynx from the oropharynx, allowing a person to breathe through the nose (Hildesheim & Levine, 1993). To allow a person to carry on respiratory functions, the nasopharynx remains open even when the surrounding muscles flexed. The nasopharynx is surrounded by salpingopharyngeal fold and tubal tonsils which are able to become inflamed when encountered infection. It contains adenoid tissue which act as protection against infection and the openings to the Eustachian tubes, which lead to the ears (Chan *et al.*, 2016).

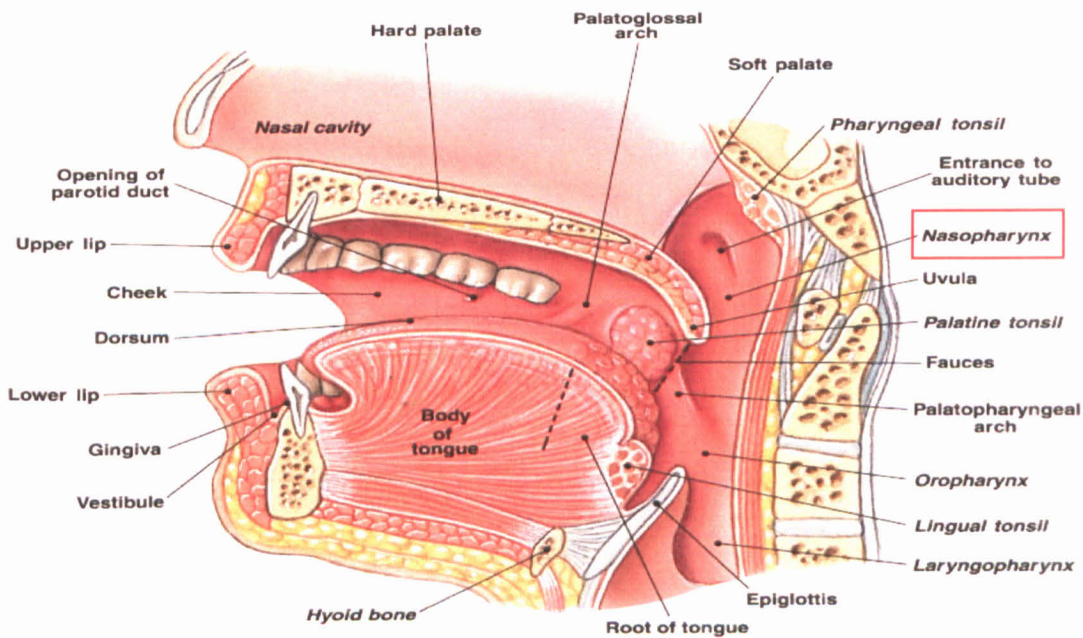


Figure 2.1a: Anatomy of nasopharynx (Adapted from <https://www.alpfmedical.info/squamous-cell/tumours-of-the-nasopharynx-introduction.html>, 2016).

2.2.1 Nasopharyngeal Carcinoma (NPC)

Nasopharyngeal carcinoma is a cancer arising mainly from the nasopharynx epithelium (Figure 2.2.1a). It is one of the types of cancer occur in the head and neck region, frequently occur at the opening of Eustachian tube in the Rosenmuller's fossa (Chan *et al.*, 2015). According to the American Cancer Society (2017), NPC is classified into 3 subtypes which are keratinizing squamous cell carcinoma, non-keratinizing squamous cell carcinoma and undifferentiated or poorly differentiated carcinoma. Some of these tumours are non-cancerous (benign) and some are cancerous (malignant). Benign tumours are rare and mostly develop in children and young adults. These tumours are not life-threatening as it does not spread to the other parts the body. In contrast, malignant tumours are able to penetrate into the surrounding tissues and spread to the other parts of the body. These malignancy in the nasopharynx is called nasopharyngeal carcinoma or NPC as abbreviation (Chan *et al.*, 2015).

NPC shows a very distinct racial and geographical distribution. In the past few years, there were approximately 65,000 new cases and 38,000 deaths happened globally (Ferlay *et al.*, 2014). There are certain populations in which these incidences are highly recorded namely, native and foreign-born Chinese, Southeast Asians (e.g. in Thailand, Philippines, Vietnam and the Bidayuh in Borneo), North Africans as well as the native of the Artic region (e.g. in Canada and Alaska) (Wee *et al.*, 2010).

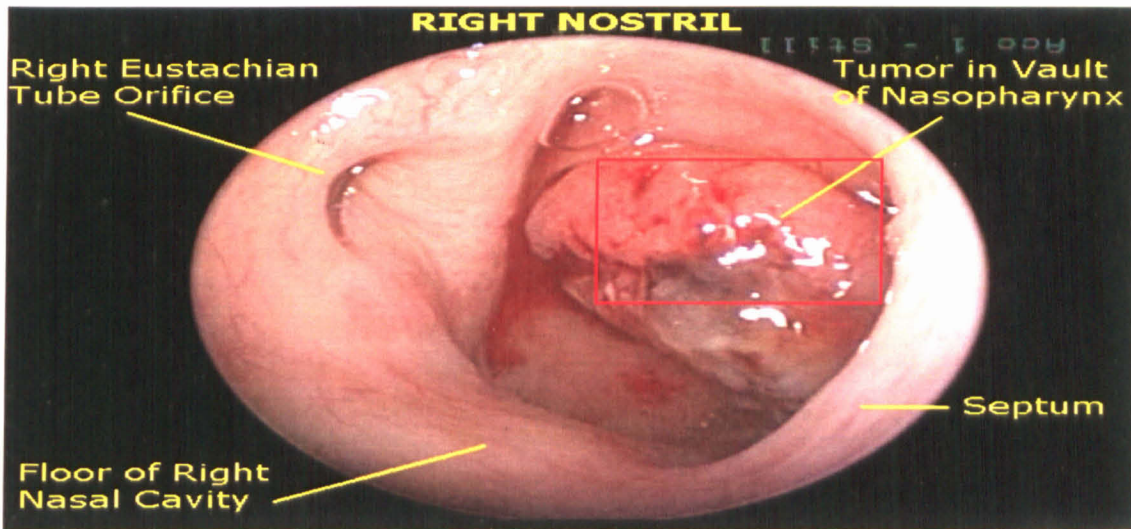


Figure 2.2.1a: Tumour occur in nasopharynx. Through the right nasal endoscopy, it can be noticed that there is nasopharyngeal tumour in the vault of nasopharynx. (Adapted from <http://www.primehealthchannel.com/nasopharyngeal-carcinoma.html>, 2012).

2.3 Aetiological Factors of Nasopharyngeal Carcinoma

2.3.1 Epstein-Barr Virus (EBV) Infection

Epstein-Barr Virus (EBV) is also known as human herpesvirus with genome composed of linear double-stranded DNA, approximately 172 kb (Young & Dawson, 2014). EBV is always present in NPC which acts as an oncogenic role. It is very common to be infected with EBV in all human population and it is asymptomatic and life-long. There is association between EBV and NPC where EBV DNA is present in the NPC cells and that the biopsy samples are clonal, arising from a single EBV infected cell (Arvin *et al.*, 2007). EBV act as the causative agent in infectious mononucleosis and is also associated with Burkitt's Lymphoma and Hodgkin's disease (Young & Dawson, 2014).

2.3.2 Environmental Factors

According to the American Cancer Society (2017), environmental factors can lead to NPC where it can be categorised into two types; the inhalatory factor and dietary factor. In other words, it is related to the human respiratory system and habits with regard to consumption

of food and drink. Eating a diet in salt-cured fish and meat are able to increase the ability of EBV to cause NPC as food preserved in this way produce chemicals which is carcinogenic and thus damage the DNA. Damaged DNA alters the cell's ability to control its growth and replication. The International Agency for Research on Cancer (IARC), has classified cigarette smoking as a risk factor for NPC as the inhalation for various types of smokes, fumes and dust may enhance the NPC to occur (Niedobitek, 2000).

2.4 Gene Study

2.4.1 Human Ribosomal Protein S8 (*uS8*) Gene

The gene targeted in this study is human ribosomal protein S8 (*uS8*) gene in NPC cell. This gene is also known as RPS8 gene. The eukaryotic ribosome is composed of 4 RNA molecules and 80 proteins. Through PCR-based cloning strategy, the DNA sequence of the entire gene is obtained. The *uS8* gene is 705 bp long and comprises of 6 exons. The start of transcription is precisely located at a C residue within 11 bp oligopyrimidine tract. The first exon contains the ATG start codon which is only 27 bp long (Davies & Fried, 1993). Kenmochi *et al.* (1998), confirmed that through the combination of PCR, Southern blotting and fluorescence *in situ* hybridization, the chromosomal location of *uS8* gene to be determined as 1p32-p34.1.

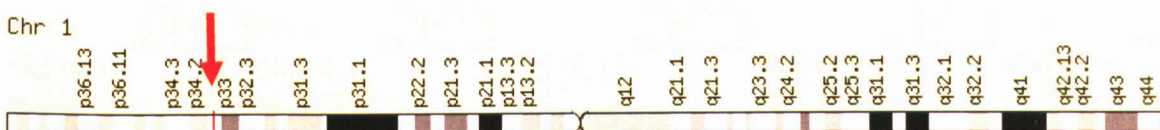


Figure 2.3: *uS8* gene in genomic location at 1p34.1, bands according to Ensembl, location according to GeneLoc. (Adapted from <http://www.genecards.org/cgi-bin/carddisp.pl?gene=RPS8>)

2.5 Diagnostic Method of NPC

Diagnostic methods of NPC include clinical evaluation of the size and location of cervical lymph nodes. Then, indirect nasopharyngoscopy will be performed to examine the primary tumour followed by neurological examination of cranial nerves. Skull erosion will also be examined via Computed tomography (CT)/magnetic resonance imaging (MRI) scan of the head and neck to below clavicles.

Also, one would undergo chest radiotherapy (anteroposterior and lateral) to see if NPC has spread to the lungs and bone scintigraphy by Tc 99 diphosphonate to show whether cancer has spread to the bones. Other diagnosis will be performed such as full blood count, urea, electrolyte, creatinine, liver function, Ca, PO₄, and alkaline phosphate. Lastly, biopsy of either the lymph nodes or primary tumour will be performed for histological examination (Chan *et al.*, 1983).

2.6 Suggested Treatments of NPC

There are three suggested treatments of NPC which are through surgery, chemotherapy or radiotherapy. For surgery, due to the anatomical position of NPC and its tendency to present with cervical lymph node metastases, it is not amenable to surgery for local control. Biopsy of the involved lymph node is the usual surgical procedure. The nasopharyngeal primary tumour is rarely biopsied. For chemotherapy, the factor taken into account in deciding the chemotherapy regimen is the efficacy. The figures for event-free survival are similar for most small chemotherapy series but therapy usually involves fairly high-dose radiotherapy to the nasopharynx (Rodriguez-Galindo *et al.*, 2005). Next, radiotherapy is given with megavoltage equipment after initial chemotherapy. A maximum dose of 45 Gy is given to the clinical target volume, which is a 1 cm margin around the MRI-detected primary site, and inferiorly down to the clavicles to include the lymph nodes (Jenkin *et al.*, 1981).

2.7 Nasopharyngeal Carcinoma (NPC) Cell Lines

The NPC cell lines are usually characterized based on their histological features and other properties such as culturing method, EBV status and patient's biodata. It is also important to know that most of the NPC cell lines are EBV negative. To date, there are more than 20 NPC cell lines that has been established (Gullo, 2008). In this project, one NPC cell line were being used namely HK1 cell line.

HK1 cell lines is established from a 59 years old male Chinese patient with histological feature of well differentiated squamous cell carcinoma (Huang et al., 1980). The patient was treated with radiation therapy by using biopsy procedure and the tissue taken out was from a recurrent tumour. HK1 cell line has a negative EBV status. The cell line can be maintained in RPMI-1640 medium supplemented with 15% fetal calf serum; 100 IU/ml penicillin and 100 mg/ml streptomycin.

CHAPTER 3: METHODOLOGY

3.1 Materials, Apparatus and Tools

Table 3.1a: Materials

Materials	Source (Brand)
6X Loading Dye	Fermentas, USA
GoTaq DNA polymerase	Fermentas, USA
dNTP Mix	Promega, UK
25 mM MgCl ₂ solution	Promega, UK
5X Green GoTaq Flexi buffer	Promega, UK
1kb Gene Ruler DNA Ladder Mix	Fermentas, USA
75% ethanol	-
dH ₂ O	-
1X TAE Buffer	-
EtBr Solution	-
Nuclease Free Water	-

Table 3.1b: Apparatus

Apparatus	Source (Brand)
Polymerase Chain Reaction (PCR) machine	SensoQuest GmbH, Germany
Nyx Technik Voltronyx – Model 37	Global Medical Instrumentation (GMI), USA
Weighing Machine	Setra, USA
UV transilluminator	-

Table 3.1c: Tools

Tools	Source
NCBI primer BLAST	National Center for Biotechnology Information (NCBI)
Tm Calculator	OligoCalc (USA)
OligoAnalyzer 3.1	Integrated DNA Technologies, USA

3.2 Cell Line Sample

In this study, the human nasopharyngeal carcinoma cell lines were used. The original source and consent for use of the cell line provided by University of Hong Kong (Hong Kong) and stored in the Immunology Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. The HK1 cell line has a histological feature of well differentiated squamous cell carcinoma. The HK1 cell line was obtained and prepared by the Immunology Lab, Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. The cDNA was also synthesised and prepared by the Immunology Lab.

3.3 Primer Design

For the use of polymerase chain reaction (PCR), using the NCBI primer blast, a set of primers which consists of forward and reverse primer were designed following all the parameters required such as the melting temperature (T_m), annealing temperature, GC content, GC clamp, primer secondary structures and product length. Then, the parameters were checked by using Oligonucleotide properties calculator (OligoCalc, USA) and Oligonucleotide analyser tool (OligoAnalyzer 3.1, Intergrated DNA Technologies, USA). These tools were used to detect if there is any potential formation of primer secondary structures. It is a

precaution steps taken to maintain the availability and specificity of primers in the PCR process.

3.3.1 Identification of Primer Binding Site

It is important to know the exact priming specificity at the DNA sequences for amplification by genes of interest: uS8 gene or also known as Homo sapiens ribosomal protein S8 (RPS8).

Homo sapiens ribosomal protein S8 (RPS8), mRNA

NCBI Reference Sequence: NM_001012.1

GenBank FASTA

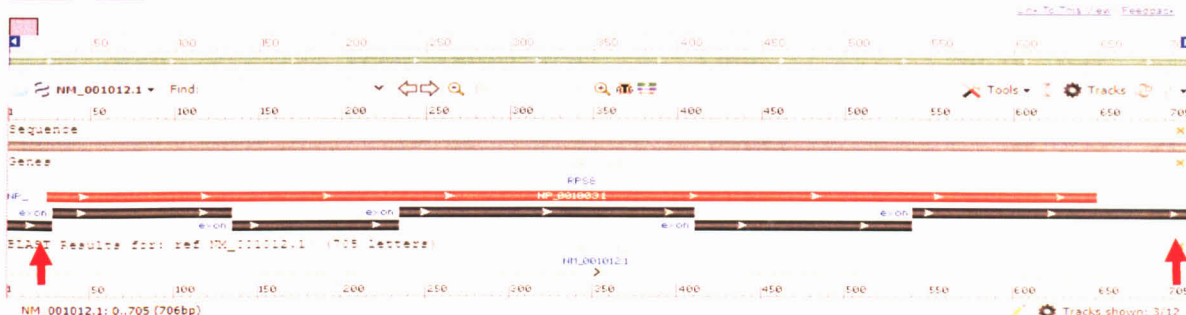


Figure 3.3.1a: Binding sites of primer pairs to the targeted cDNA (NCBI, USA)

3.4 Gradient PCR using RPS8 Primer

Gradient PCR was conducted using the designed primers which is specific to the target gene, PCR. It was conducted to determine the best optimal annealing temperature. A Master Mix was prepared in a 1.5 ml sterile microcentrifuge tube as follows (Table 3.4a):

Table 3.4a: 6X Master Mix for gradient PCR using RPS8 primer

Reagents	1X Master Mix (µL)	6X Master Mix (µL)
Green GoTaq Buffer	5.0	30.0
MgCl ₂	1.0	6.0
dNTPs mix	0.5	3.0
GoTaq DNA Polymerase	0.125	0.75
Nuclease Free Water	15.375	92.25
Total volume	22.0	132.0