UNIVERSITI PUTRA MALAYSIA

CLONING OF HUMAN- INTERLEUKIN-12 INTO pJET CLONING VECTOR FOR ANTI-CANCER VACCINE DEVELOPMENT

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DECLARATION

This is to certify that the project entitled " Cloning of human Interleukin-12 (hIL-12) into pJET Cloning Vector for Anti-cancer Vaccine Development" was completed and submitted to the Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences by Muhamad Alhapis bin Che Ani in partial fulfillment for the requirement of the degree of Bachelor of Science (Hons.) from Universiti Putra Malaysia.

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بِسْمِ اللهِ الرَّحْمَنِ الرَّحِيْمِ

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LIST OF ABBREVIATION

ND	Newcastle disease	$CaCl_2$	Calcium chloride
SDS	Sodium dodecyl sulfate	kb	Kilo base pair
NDV	Newcastle disease virus	bp	Base pair
E. coli	*Escherichia coli*	NaOH	Sodium hydroxide
hIL-12	Human interleukin-12		
RNA	Ribonucleic acid		
mRNA	Messenger ribonucleic acid		
DNA	Deoxyribonucleic acid		
kDa	Kilodalton		
AGE	Agarose gel electrophoresis		
PCR	Polymerase chain reaction		
LB	Luria-Bertani		
OD	Optical density		
^{o}C	Degree celcius		
µl	Microliter		
ml	Mililiter		
µg/ml	Microgram per mililiter		
mM	Milimolar		
M	Molar		
nm	Nanometer		
rpm	Rotation per minute		
EDTA	Ethylenediaminetetraacetic acid		

ABSTRACT

The Newcastle disease virus can replicate rapidly and kill human cancer cells. Therefore it has the potential to be developed as a cancer vaccine. However, the immune system hinders the replication of the virus in these cells. In order to make it more efficient as a cancer vaccine, the the human interleukin-12 (hIL-12) gene will be cloned into the virus. However, before this gene can be transferred to the virus, it must first be cloned into a cloning vector. In this project, the hIL-12 gene was obtained from the plasmid pUNO-hIL12 and cloned into a pJET cloning vector. A set of forward and reverse primers with *NheI* restriction included was designed to amplify hIL-12 gene. This will lead to producing the hIL-12 gene fragment with *NheI* restriction sites after amplification by polymerase chain reaction (PCR) cycles. The PCR product was then purified by using MEGAquick-spin™ Total Fragment DNA Purification Kit. The purified hIL-12 was then cloned into pJET cloning vector by T4 DNA ligase and transformed into competent *Escherichia. coli* Top 10 cells by using heat-shock transformation method. The cloning was verified using *NheI* restriction enzyme digestion analysis and further confirmed by sequencing.

ABSTRAK

Penyakit sampar ayam (NDV) boleh membiak dengan cepat dan membunuh sel-sel kanser manusia. Oleh itu, ia mempunyai potensi untuk dibangunkan sebagai vaksin kanser. Walau bagaimanapun, sistem imun menghalang replikasi virus dalam sel-sel. Untuk menjadikannya lebih cekap sebagai vaksin kanser, interleukin-12 daripada manusia (HIL-12) gen akan diklon ke dalam virus. Walau bagaimanapun, sebelum gen ini boleh dipindahkan kepada virus, ia mesti diklon ke dalam vektor pengklonan terlebih dahulu. Dalam projek ini, hIL-12 gen telah diperolehi daripada plasmid pUNO-hIL12 dan telah diklon ke dalam vektor pengklonan pJET. Satu set primer kehadapan dan primer kebelakang dengan sekatan tapak sekatan *Nhe*I dimasukkan bagi memperbanyakan gen hIL-12. Dengan ini, fragmen hIL-12 gen dengan tapak sekatan *Nhe*I akan terhasil oleh tindak balas berantai polimerase (PCR). Produk daripada PCR ini kemudiannya ditulenkan dengan menggunakan "MEGAquick-spin™ Total Fragment DNA Purification Kit". hIL-12 yang telah ditulenkan akan diklon ke dalam vektor pengklonan pJET oleh T4 DNA ligase dan ditransformasikan ke dalam kompeten sel-sel *Escherichia. coli* Top 10 dengan menggunakan kaedah transformasi kejutan-haba. Pengklonan ini telah disahkan menggunakan kaedah penghadaman enzim pembatas *Nhe*I dan seterusnya disahkan oleh penjujukan DNA.

CHAPTER 1

INTRODUCTION

Cancer is a disease in which the cells in malignant tumors divide uncontrollably and consequently will invade the surrounding cells and tissues, while in more severe cases will lead to metastasis. It is a consequence from either genetic or environmental factors (Al-qubaisi *et al.*, 2011). Data from the National Cancer Institute, the Centers for Disease Control and Prevention, North American Association of Central Cancer Registries and National Center for Health Statistics stated that 1,665,540 new cancer cases and 585,720 cancer deaths are projected to occur in the United States in 2014 (Siegel *et al.,* 2014). Meanwhile, report from International Agency for Research on Cancer (IARC) Globocan of the World Health Organisation (WHO) stated that the prevalence of cancers in Malaysia has rose from 32 000 cases in 2008 to 37 000 in 2012. The mortality rate due to cancer stood at 20 100 death in 2008 and has increased to 21 700 deaths in 2012. The most common cancers among Malaysians are breast, colorectal, lung, cervix and nasopharynx cancers (Lam *et al.*, 2011).

The common approaches used in treating cancer are radiotherapy, chemotherapy and surgical removal of the tumor. However they are not the best approach as there might be severe side effects (Lam *et al.*, 2011). Thus, new alternative approaches towards the treatment of cancer are being developed. For example, the Newcastle disease virus (NDV) has been studied as prominent candidate to treat cancer in

virotherapy. NDV has been identified to have great potential as an effective cancer vaccine due to its proven ability to selectively kill human cancer cells (Reichard *et al.*, 1992). Studies conducted have proven that NDV can effectively eliminate colorectal cancer cells, MCF-7 human breast carcinoma cell, murine myelomonocytic leukemia and human promyelocytic leukemia (HL60) and human T-lymphoblastic leukemia (CEM-SS) cells (Chia *et al.,* 2014; Ghrici *et al.,* 2013; Alabsi *et al,* 2012; Abu Bakar *et al.,* 2012). However, in order to improve the specificity and efficacy of NDV towards cancer cells, the NDV genome will be modified to carry human interleukin-12 (hIL-12) gene to elicit anti-cancer immune response. The hIL-12 is a cytokine that bridges the innate and adaptive immunity while stimulating the production of IFN – γ (Jakobisiak *et al.,* 2003).

In this study, the hIL-12 gene sequence with *NheI* restriction sites will be cloned into pJET cloning vector. The *NheI* restriction sites were introduced in the primers used to amplify hIL-12 gene. Then, the modified hIL-12 gene will be produced through polymerase chain reaction (PCR) before being cloned into pJET cloning vector. The cloned plasmids will be transformed into *Escherichia coli.*

The objective of this study is to successfully clone the hIL-12 into pJET cloning vector which later will then be used in future vaccine construction of NDV.

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