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BACHELOR OF SCIENCE (HONS.)

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By

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Thesis submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Bachelor of Science (Hons.) Cell and Molecular Biology

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Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement for the degree of Bachelor of Science (Hons.) Cell and Molecular Biology

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Chair: Dr. Nurulfiza Mat Isa

Faculty: Faculty of Biotechnology and Biomolecular Sciences

pNZ:vig vector is a bicistronic vector isolated from *Lactococcus lactis* that carrying VP2 gene and GFP expressed under IRES-mediated translation. IL-2 cytokines is a molecule produce by T-helper cells in response to antigen attack. Despite such drawbacks of conventional live vaccines, it has potential of utilize food grade *Lactococcus lactis* as a live vector for the delivery of vaccine. To obtain a natural vaccine-enhancing molecule, in order to stimulate the proliferation of T-cells in chicken infected with IBDV, partial IRES-IL2 gene was successfully designed to introduce into pNZ:vig vector. This combination covers the requirement to improve the potency of the immune response against IBDV. In this study, GFP cassette was removed from the vector to clone IL-2 cytokine gene. A commercial vector pIDT (Clontech) that carry partial IRES-IL2 was maintained and propagated by using *Escherichia coli* TOP10. Positive transformants were confirmed by using colony PCR and concurrently validated further by agarose gel electrophoresis analysis. Forward and reverse primers were designed to amplify partial IRES-IL2 fragment by using PCR. Gel electrophoresis confirmed the estimated size of the partial IRES-IL2 fragment was approximately 700 basepairs. Meanwhile, pNZ:vig vector was

extracted from *L.lactis* NZ9000 by plasmid extraction and subjected to restriction enzyme digestion by using restriction enzymes *Kpn1* and *Nar1*. Digested pNZ:vig vector was verified by agarose gel electrophoresis and the results depicted the expected size of the vector was approximately 6000 basepairs. Restriction enzyme digestion for pIDT plasmid needs further optimization to obtain partial IRES-IL2 fragment. The ligation of partial IRES-IL2 fragment into pNZ:vig vector was not successful thus needs further optimization.

Keywords : IL-2 cytokine, VP2, Bicistronic vector

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk Ijazah Sarjana Muda Sains (kepujian) Biologi Sel dan Molekul

PENGKLONAN GEN CYTOKIN IL-2 DARIPADA AYAM KE DALAM pNZ:vig VEKTOR

OLEH

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pNZ:vig vektor ialah vektor bicistronic yang diasingkan daripada *Lactococcus lactis* yang membawa gen VP2 dan GFP menggunakan ekspresi dengan bantuan elemen IRES. IL2 cytokin ialah molekul yang dihasilkan daripada sel T pembantu kesan daripada tindak balas terhadap serangan antigen. Vaksin konvensional yang digunakan sebelum ini mempunyai beberapa kekurangan telah membuka prospektif bagi menggunakan gred makanan *Lactococcus lactis* sebagai vektor hidup untuk penghantaran vaksin. Bagi mendapatkan molekul semulajadi untuk penambahbaikan vaksin, untuk meransang pembahagian sel T bagi ayam yang dijangkiti IBDV, gen separa IRES-IL2 telah berjaya direka untuk diklonkan ke dalam vektor pNZ:vig. Pengklonan ini telah meliputi kehendak untuk penambahbaikan potensi untuk menghasil tindak balas immune terhadap IBDV. Dalam pembelajaran ini, GFP gen telah dikeluarkan daripada vector untuk mengklon gen IL2 cytokin. Vektor komersial pIDT (Integrated DNA Technology) yang membawa gen separa IRES-IL2 dikekalkan dan dibiakkan menggunakan *Escherichia coli* TOP10. Transforman yang berjaya telah disahkan menggunakan

PCR (tindak balas rantaian polymerase) koloni dan diikuti dengan pengesahan selanjutnya menggunakan elektroforesis gel. Primer hadapan dan belakang telah direka bagi memperbanyakkan serpihan separa IRES-IL2 menggunakan PCR (tindak balas rantaian polymerase). Elektroforesis gel mengesahkan anggaran saiz separa IRES-IL2 adalah 700 pasangan bas. Sementara itu, vektor pNZ:vig telah diasingkan daripada *L. lactis* NZ9000 menggunakan kaedah pengasingan plasmid dan telah digunakan untuk penghadaman enzim retriksi menggunakan enzim retriksi *Kpn1* and *Nar1*. Vektor pNZ:vig yang telah dihadam diverifikasikan menngunakan gel elektroforasi dan keputusannya menggambarkan saiz vektor yang dijangkakan iaitu dalam anggaran 6000 pasangan bas. Penghadaman enzim retriksi bagi plasmid pIDT perlu diperbaiki selanjutnya bagi mendapatkan serpihan separa IRES-IL2. Ligasi bagi serpihan separa IRES-IL2 yang tidak berjaya perlu diperbaiki selanjutnya bagi membolehkan ligasi ke dalam vektor.

Kata Kunci : IL-2 cytokine, VP2, Vektor bicistronic

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APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of the requirement for the degree of Bachelor of Science (Hons.) Cell and Molecular Biology. The member of Supervisory Committee was as follows:

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Date:

DECLARATION

Declaration by undergraduate student

I hereby confirm that:

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Declaration by Supervisor

This is to confirm that:

- The research conducted and the writing of this thesis was under supervision.

Signature: _____

Name of Supervisory Committee: Dr. Nurulfiza Mat Isa

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LIST OF ABBREVIATIONS

IL2	Interluekin-2
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
RE	Restriction enzyme
PCR	Polymerase chain reaction
LB	Luria Bertani media
GM17	M17 media supplemented with 0.5% glucose
SGM17	M17 media supplemented with 0.5% glucose and 0.5M sucrose
TE / TAE Buffer	Tris-CI EDTA / Tris-acetate EDTA
$MgCl_2$	Magnesium chloride
$CaCl_2$	Calcium chloride
bp	Base pairs
kb	Kilo base
v/v	Volume per volume
w/v	Weight per volume
µM/ mM/ M	Micro molar/ Millimolar/ Molar
µl/ ml	Microliter/ Milliliter
rpm	Rotation per minute
g	Relative centrifugal force (RCF)
GRAS	Generally recognized as safe
LAB	Lactic acid bacteria

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CHAPTER 1

INTRODUCTION

Lactococcus lactis is a type of Gram positive lactic acid bacteria (LAB) which has been widely used mainly in fermentation process in food industry. LAB has been graded Generally Recognized as Safe (GRAS) status. Despite of their well-known food-grade bacteria, researchers had also shown their positive prospective in pharmaceutical field as vaccines and drugs production (Guchte, Kok, and Venema, 1992). Lack of ability to form colonies inside the digestive tract and non-pathogenic to human are the large potential possess by *L. lactis* as a vaccine antigen delivery vector (Mercenier, 2000).

Infectious bursal disease virus (IBDV) is the causative agent of Gumboro disease that caused high morbidity and mortality primarily in 3-6 weeks old chicken (Wyeth and Cullen, 1976). The infection of IBDV caused immunosuppression in chicken makes susceptible to other opportunistic disease and interferes with effective vaccination (Allan et al., 1972; Giambrone et al., 1977; Jen and Cho, 1980).

Conventional attenuated viral vaccine associated with the risks make it indispensable in the development of vaccines subunit which is safer and effective (Pradhan et al., 2012). In addition, soma variant of IBDV live vaccines were not able to confer good protection against IBDV hence caused immunosuppression and sub-clinical disease (Fussell, 1998; Mazariegos et al., 1990). VP2 is the major host-protective immunogen of IBDV that contains independent epitopes as antigenic regions which responsible for the induction of neutralizing antibody production has been seen as positive prospective to develop efficient and safe vaccines subunit

(Heine et al., 1991). The efficacy of VP2 immunodominant fragment have been reported which can induce both humoral and cellular immunity against infectious bursal disease (Pradhan et al., 2012).

pNZ:vig is a newly developed bicistronic vector which can co-express multiple genes in eukaryotic system with the presence of IRES element. This newly constructed plasmis is a modified plasmid from plasmid DNA isolated from lactic acid bacteria (LAB) which is *Lactococcus lactis* (Mutalib et al., 2014). Chicken IL2 cytokines are natural modulators of immune system that can act as natural vaccine-enhancing molecule towards IBDV disease hence further improve the immune response of conventional vaccines towards avian pathogen. Previous study has shown that VP2 and IL2 vaccinated chicken were better protected compared to VP2 alone. (Hulse and Romero, 2004)

Therefore, the objective of this study was to clone chicken IL2 cytokine gene into pNZ:vig vector. Partial IRES-GFP was removed from pNZ:vig plasmid DNA to clone partial IRES-IL2 gene into the plasmid. The partial IRES-IL2 fragment need to be ligated to pNZ:vig vector to develop a newly constructed plasmid which co-express VP2 capsid protein and IL2 cytokine gene via IRES element towards an efficient and safe DNA-mediated vaccination.

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