

### **UNIVERSITI PUTRA MALAYSIA**

### CLONING OF EUKARYOTIC EXPRESSION CASSETTE INTO PMG36E LACTOCOCCAL VECTOR

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# CLONING OF EUKARYOTIC EXPRESSION CASSETTE INTO pMG36e LACTOCOCCAL VECTOR



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BACHELOR OF SCIENCE (HONS.) CELL AND MOLECULAR BIOLOGY

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### CLONING OF EUKARYOTIC EXPRESSION CASSETTE INTO pMG36e LACTOCOCCAL VECTOR



By

LIM YI YI

Thesis submitted to the Department of Cell and Molecular biology, Faculty of Biotechnology and Biomolecular Sciences, in Fulfilment of the requirement for Degree of Cell and Molecular Biology.

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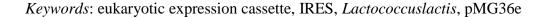
Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement of the degree of Cell and Molecular Biology.

Cloning of Eukaryotic Expression Cassette into pMG36e Lactococcal Vector

By Lim Yi Yi June 2015

### Dr. Nurulfiza Mat Isa, PhD Faculty of Biotechnology and Biomolecular Sciences

Lactococcuslactis(L. lactis) is a Gram-positive bacteria traditionally used in food industry for flavouring and fermenting processes. In recent years, L. lactis was found to be able to deliver plasmid DNA into eukaryotic cells and express heterologous protein, which makes it being developed as safer alternative for vaccination. The increasing demand of L. lactisbeyond application in food industry raises the need to develop lactococcal bicistronic vector with target gene expression in the eukaryotic system. The objective of this study is to construct a lactococcal vector containing modified eukaryotic expression cassette, with internal ribosome entry site (IRES) inserted between CMV promoter and Poly A signal to allow transcription and translation of two proteins in a single cassette. IRES sequence was PCR-amplified from pRetroX-IRES-ZsGreen1 vector, digested with *Eco*RV and *XhoI*, and ligated into eukaryotic expression cassette from pcDNA 3.1 HisA. Constructed vector was transformed into E. coli TOP 10 and the orientation of IRES fragment in the modified eukaryotic expression cassette was confirmed by DNA sequencing. The modified eukaryotic expression cassette was PCR-amplified from pcDNA3.1HisA/IRES before sub-cloning into lactococcal vector pMG36e.pMG36eharbouringmodified eukaryotic expression cassette was successfully transformed into E. coli TOP 10 but not L. lactis NZ9000. Optimization of transformation protocol for L. lactis NZ9000 using electroporation is needed to enhance transformation efficiency. Segragational instability of constructed vector in E. coli host was detected, which may have been caused by production of single-stranded intermediates and high-molecular weight DNA due to rolling-cycle replication of the pMG36e vector.



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Abstrak thesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk ijazah Sel dan Molekula Biologi.

Pengklonan Kaset Pengekspresan Eukariot ke dalam Vektor Lactococcal

pMG36e Oleh Lim Yi Yi June 2015

### Dr. Nurulfiza Mat Isa, PhD Fakulti Bioteknologi dan Biomolekular Sains

*Lactococcus lactis (L. lactis)* merupakan bakteria Gram-positif yang digunakan untuk proses penambahan perisa and fermentasi dalam industry makanan. Kebelakangan ini, kebolehan L. lactis untuk menyalurkan DNA plasmid ke dalam sel eukariot dan ekspres protein "heterologous" telah dijumpai, seterusnya menjadikan L. lactis sebagai alternatif yang selama tuntuk vaksinasi. Pemintaan tinggi L. lactis dalam pelbagai bidang selain daripada industry pemakanan telah merangsang pembanguan vector lactococcal "bicistronic" yang mensasarkan ekspresi gen di dalam system eukariot. Objektif kajian ini adalah untuk membina vector lactococcal yang mempunyai kaset pengekspresan eukariot yang diubahsuai dengan pengisian tapak kemasukan ribosomd alaman (IRES) di antara promoter CMV dan isyarat Poly A untuk transkripsi dan pengekspresan dua jenis protein dalam satu kaset. Jujukan IRES telah diamplifikasi dengan saringan PCR dari vektor pRetroX-IRES-ZsGreen1, dipotong dengan EcoRV and *XhoI*, dan ditampalkan ke dalam kaset pengekspresan eukariot dari pcDNA 3.1 HisA.Vektor yang telah dibina ditransform ke dalam bacteria E.coli dan orientasi jujukan IRES dalam kaset pengekspresan eukariot yang diubahsuai telah disahkan dengan penjujukan DNA. Kaset pengekspresan eukariot yang telah diubahsuai diamplifikasi dengan saringan PCR dari pcDNA3.1HisA/IRES sebelum pengklonan semula ke dalam pMG36e. Rekombinan pMG36e telah berjaya ditransform ke dalam bacteria E. coli tetapi tidak berjaya pada L. lactis. Pengubahsuaian protocol transformasi L. lactis perlu dioptimumkan. Ketidakstabilan "Segregational" oleh rekombinan pMG36e telah dikesan dalam perumah bacteria E. coli yang mungkin disebabkan produksi bebenang tunggal pengantara dan juga berat DNA molekul yang tinggi akibat replikasi gulungan kitaran pMG36e.

Keywords: kasetpengekspresaneukariot, IRES, Lactococcuslactis, pMG36e

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#### Declaration

Declaration by undergraduate student

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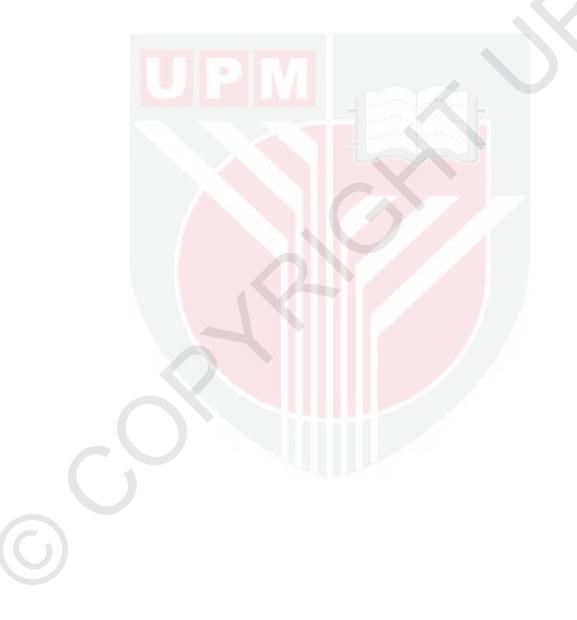


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### LIST OF ABBREVIATIONS

bp	Base pairs
DNA	Deoxyribonucleic acid
EDTA	EthylenediamineTetraacetic Acid
E. coli	Escherichia coli
GM17	M17 supplemented by 0.5% (w/v) glucose
HCl	Hydrochloric acid
IRES	Internal ribosome entry site
L. lactis	Lactococcuslactis
LB	Luria Bertani
ng	Nanogram
OD	Optical density
PCI	Phenol:chloroform: isoamyl alcohol
PCR	Polymerase Chain Reaction
PolyA	Polyadenylation
RNA	Ribonucleic acid
SGM17	M17 supplemented by 0.5% glucose and 0.5M sucrose
TE	Tris-EDTA (EthylenediamineTetraacetic Acid)
Tris	Tris(hydroxymethyl) aminomethane
μL	Micoliter
μΜ	Micromolar

#### **CHAPTER 1: INTRODUCTION**

Lactococcus lactis (L. lactis) is a Gram-positive coccus from the lactic acid bacteria group with novel properties for application in the preservation and flavor production of food. Due to their contribution in the food industry, L. lactis has been designated as generally recognized as safe (GRAS) organism (Pontes *et al.*, 2011). The nonpathogenic characteristic coupled with the ability to express heterologous proteins rendered L. lactisthe ideal choice for safe production of antigens and medically important proteins (De Ruyter *et al.*, 1996). In a more recent development, L. lactis was found to be able to invade and deliver plasmid DNA encoding proteins into the eukaryotic cells. Thus, there is growing interest in developing L. lactis as bacterial delivery system for antigens as part of safer alternatives of vaccination.

The wider application of *Lactococcus* bacteria beyond the food industry mediates the need for development of lactococcal vectors. There are less commercially available vectors for *L. lactis* compared to vectors for *E. coli*. Besides, a lactococcal vector usually consists of transcriptional and translational structures recognized by only prokaryotic host. For it to be expressible in eukaryotic host, the existing lactococcal vector usual be modified to carry eukaryotic transcription and translation features.

Traditionally, co-transfection of two different vectors; one carrying reporter protein and another carrying protein of interest, is needed for most delivery of vectors into mammalian cells. The transfection of the protein of interest which is presumed to be successful with the successful transfection of the reporter protein is unreliable and unpredictable (Hunt, 2005). Internal ribosome entry site (IRES) elements are able to internally initiate the translation of RNA and facilitate the expression of two or more proteins. When modified into expression vectors, these bicistronic expression vectors are able to translate the first gene in a cap-dependent manner and the second in IRES dependent manner (Vagner *et al.*, 2001). Using IRES-incorporated bicistronic expression vector, the selection efficiency during transgenesis improved, as over 90% of cells expressing the selectable marker also express the gene of interest.



Therefore, the objective of this study was to construct lactococcal vector pMG36e containing eukaryotic expression cassette. The eukaryotic expression cassette was first created by amplification of IRES fragment using polymerase chain reaction before cloning into the expression cassette of pcDNA 3.1 HisA. The expression cassette is then sub-cloned into pMG36e using the same cloning strategy and transformed into *L. lactis*. The added features enabled the expression of heterologous proteins in both eukaryotic and prokaryotic host while the prokaryotic backbone ensured continuous propagation of the vector in prokaryotic host. IRES incorporated enable the expression of multiple proteins in the host.

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#### APPENDICES