

**MOLECULAR CLONING OF
TWO VIRULENCE GENES FROM**

Agrobacterium tumefaciens

By

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ABSTRACT

MOLECULAR CLONING OF TWO VIRULENCE GENES

FROM *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens causes tumour formation, known as crown gall, in plants. This is due to the ability of the bacterium in transferring a portion, known as T-DNA, from the tumour-inducing (Ti) plasmid, to the host cell nucleus. Hence, *A. tumefaciens* has been widely used as a genetic transformation tool. The virulence (*vir*) genes encode the Vir proteins, which are responsible for the translocation of the T-DNA. VirE2 and VirD2 proteins are involved in the cleavage of T-DNA from the Ti plasmid and translocation of the T-DNA-protein complex to the host cell nucleus. Therefore, the VirE2 and VirD2 proteins play key roles in determining the efficiency of the genetic transformation process. The main objective of this study is to separately clone *virD2* and a non-translational fusion *virD2* and *virE2* genes into an expression vector pING2, thereby increasing the efficiency of the *Agrobacterium*-mediated transformation by increasing the expression of the key virulence genes. The *virD2* gene was successfully subcloned from the cloning vector into the *Agrobacterium* expression vector pING2. The *virE2-virD2* fusion gene construct contains an intervening ribosomal-binding site

(RBS), to facilitate efficient translation of *virD2*. For generating the fusion gene construct, first, the two *vir* genes were separately amplified by polymerase chain reaction (PCR). The RBS sequence in the PCR-amplified *virD2* was generated by including the RBS sequence in the 5' primer. A complementary sequence was inserted to the 3' end of *virE2* and 5' end of *virD2* to facilitate the fusion of PCR-amplified *virE2* and *virD2* fragments. The Gibson assembly method produced the desired fusion gene construct DNA fragment, but the yield was insufficient for further cloning experiment. This fusion fragment requires further amplification. For future studies, the expression vector pING2 carrying *virE2*, *virD2* and *virE2-virD2* will be transferred into *A. tumefaciens*, where their effects on *Agrobacterium*-mediated transformation efficiency can be evaluated.

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DECLARATION

I hereby declare that the project is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

GOH HUI TING

APPROVAL SHEET

This project report entitled **“MOLECULAR CLONING OF TWO VIRULENCE GENES FROM *Agrobacterium tumefaciens*”** was prepared by GOH HUI TING and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (HONS) Biotechnology at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **GOH HUI TING** (ID: **11ADB04063**) has completed this final year project entitled “MOLECULAR CLONING OF TWO VIRULENCE GENES FROM *Agrobacterium tumefaciens*” supervised by Assoc. Prof. Dr. Wong Hann Ling from the Department of Biological Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(GOH HUI TING)

TABLE OF CONTENT

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
DECLARATION	v
APPROVAL SHEET	vi
PERMISSION SHEET	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xv

CHAPTERS

1	INTRODUCTION	1
	1.1 <i>Agrobacterium</i> genus	1
	1.2 <i>Agrobacterium</i> -mediated Transformation	2
	1.3 Significance and Objectives of the Project	4
2	LITERATURE REVIEW	5
	2.1 Tumour Inducing (Ti) Plasmid	5
	2.2 Virulence (Vir) Proteins	7
	2.2.1 VirA and VirG	7
	2.2.2 VirD2 and VirE2	8
	2.3 Polymerase Chain Reaction (PCR)	9
	2.3.1 Overlap Extension PCR	11
	2.4 Gateway® Cloning Technology	13
	2.5 Gibson Assembly Cloning	14
	2.6 <i>Agrobacterium</i> Expression Vector, pING2	16
3	METHODS AND MATERIALS	18
	3.1 List of Materials and Apparatus Used	19
	3.2 Culture Medium and Solution Preparation	21
	3.2.1 Preparation of 2x Yeast Extract and Tryptone (YT) Medium	21
	3.2.2 Preparation of 10x Tris/acetate/EDTA (TAE) Buffer	21

	3.2.3	Preparation of TE Buffer	21
	3.3	Preparation of Antibiotics Stock	22
	3.4	Preparation of Electrocompetent Cells	22
	3.5	Extraction of Plasmid Using Miniprep Kit	23
	3.6	Recovery of Bacterial Strain <i>Escherichia coli</i> DH5 α	24
	3.7	Gel Electrophoresis	24
	3.8	Modification of <i>virD2</i> and <i>virE2</i> Genes	25
	3.8.1	Addition of Ribosomal Binding Site onto <i>virD2</i> and <i>virE2</i> Genes	25
	3.8.2	Overlap Extension PCR to fuse <i>virD2</i> and <i>virE2</i> genes	27
	3.8.3	Gibson Assembly Master Mix Method	29
	3.9	LR Recombination to Clone <i>virD2</i> into pING2	30
	3.10	Screening for Transformed Colonies	31
4		RESULTS	33
	4.1	Electrocompetent Cells Competency Analysis	33
	4.2	Extraction of Plasmid pENTR- <i>virD2</i> and pENTR - <i>virE2</i>	34
	4.3	RBS Insertion into <i>virE2</i> gene and <i>virD2</i> genes	36
	4.4	Overlap Extension PCR	38
	4.5	Fusion of Two Fragments by Using Gibson Assembly Method	39
	4.6	Propagation and Extraction of vector pING2	40
	4.7	Screening for Plasmid of Interest by Colony PCR	41
5		DISCUSSION	43
	5.1	Electrocompetent cells <i>Escherichia coli</i> TOP10	43
	5.2	Isolation of Plasmid DNA	44
	5.3	RBS Insertion and Amplification of <i>virD2</i> and <i>virE2</i> Genes	45
	5.4	Overlap Extension PCR	47
	5.5	Gibson Assembly to Fuse <i>virE2</i> -RBS and RBS- <i>virD2</i>	48
	5.6	Propagation of Expression Vector, pING2	50
	5.7	Subcloning of <i>virD2</i> gene into pING2	51
	5.8	Screening of Plasmid Containing <i>virD2</i> Gene	53
	5.9	Future Studies	54
6		CONCLUSION	55
		REFERENCES	56

APPENDICES	62
Appendix A	62
Appendix B	63
Appendix C	64
Appendix D	65
Appendix E	66

LIST OF TABLES

Table		Page
3.1	List of apparatus and equipments and their respective manufacturers used in this project	19
3.2	List of chemicals used and their respective manufacturers	20
3.3	The concentration of antibiotics	22
3.4	Primers set for RBS insertion onto <i>virD2</i>	25
3.5	Primers set for RBS insertion onto <i>virE2</i>	25
3.6	Components for PCR mixture for <i>virD2</i> and <i>virE2</i> amplification	26
3.7	PCR condition for <i>virD2</i> and <i>virE2</i> amplification	26
3.8	Components for first stage of OE-PCR	27
3.9	Condition for first stage OE-PCR	28
3.10	Components for second stage of OE-PCR	28
3.11	Condition for second stage OE-PCR	29
3.12	Reaction mixture of Gibson assembly master mix method	29
3.13	Reaction mixture for LR recombination	30
3.14	Primers set used in colony PCR	31
3.15	Components for colony PCR	31
3.16	Conditions for colony PCR	32
4.1	Data of extracted pENTR- <i>virD2</i>	35
4.2	Data of extracted pENTR- <i>virE2</i>	35

4.3	Data of purified RBS- <i>virD2</i>	37
4.4	Data of purified <i>virE2</i> -RBS	37
4.5	Data of extracted pING2	41

LIST OF FIGURES

Figure		Page
2.1	The Ti plasmid structures	6
2.2	Agrobacterium-mediated transformation process	9
2.3	PCR process	10
2.4	Illustration of the overlap extension PCR	12
2.5	BP and LR reaction	14
2.6	One-step assembly of DNA fragments	15
2.7	The expression vector, pING2	17
3.1	Experimental workflow	18
4.1	Tha agar plate with transformed <i>E. coli</i>	33
4.2	Gel image of extracted plasmid pENTR-wild type- <i>virD2</i> and pENTR-wild type- <i>virE2</i> after agarose gel electrophoresis	35
4.3	Electrophoresis gel image of amplified RBS inserted <i>virE2</i> and <i>virD2</i>	37
4.4	Electrophoresis gel image of overlap extension (OE) PCR during stage 1	38
4.5	Electrophoresis gel image of gene fusion by Gibson assembly mix	39
4.6	Electrophoresis gel image of extracted pING2	40
4.7	Electrophoresis gel image of colony PCR for pING2- <i>virD2</i>	42

	transformed <i>E. coli</i> TOP10	
5.1	Schematic diagram of RBS insertion onto <i>virE2</i> and <i>virD2</i> genes	46
5.2	Schematic diagram of overlap extension PCR or <i>virE2</i> -RBS and RBS- <i>virD2</i> gene constructs	47
5.3	Schematic diagram of Gibson assembly to construct <i>virE2</i> -RBS- <i>virD2</i>	50
5.4	Schematic diagram showing the LR cloning reaction between pENTR- <i>virD2</i> and pING2	51