

Abstract

In this study, *PSMA* was divided into 3 fragments, G1, G2 and G3. Two-step PCR was done to construct G1 sub-divided fragments (a, b, c, d, e, and f). G2 and G3 were amplified using specific primers and the obtained PCR products were subsequently purified and proceed for cloning.

In order to clone each *PSMA* fragment, each PCR amplified fragment were ligated into pGEM-T vector and transformed into JM109 competent cells, which provided the complete sequences of G2 and G3; with some minimum mutations in G1.

Overlapping extension PCR was conducted to combine the fragments; so the G1 fragment was amplified from the isolated plasmid and G2-3 overlapped fragment was amplified using the plasmid isolated sample. PCR optimization was carried out after overlapping of G1 and G2-3, and the fragments were purified. Overlapping of *PSMA* G1 with G2-3 was carried out and a faint band was produced.

The purified product was subsequently cloned using pGEM®-T vector; the recombinant construct was isolated by plasmid isolation and then digested with restriction enzymes such as *SfiI* and *NotI*. It was then purified and ligated into expression vector pPICZ α A, which had been predigested with *sfiI* and *NotI*. Later on, it was transformed into Top10 competent cells. This recombinant construct (pPICZ α A-*PSMA*) was isolated and linearized with restriction enzyme *SacI*.

Acknowledgement

I would like to express my gratitude to Allah (God) for giving me His blessings and providing me with strength to complete this work.

Special thanks go to King Abdullah Bin Abdul Aziz Al-Saud for giving me this study opportunity by supporting me with a scholarship.

I would also like to express my utmost gratitude to my supervisor Dr. Zulqarnain Mohamed for his patience in guiding me. His guidance, understanding and advice throughout the whole study lead to completion of this work, which is presented here and is most appreciated.

My sincere thanks to my senior Teh Ser Huy for her patience, and continuous guidance as well as advices to equip me with the skill to carry out basic genetic lab work. I appreciate her step by step guidance and the knowledge that she has equipped me. I would also like to take this opportunity to thank Hussin Alwan and Fatemeh Shahhosseini for teaching me basic genetic procedures.

I would also like to thank my colleague in the genetics lab Jameel and Johnson for sharing their experiences and knowledge as well as providing help throughout this study.

My thanks also goes to my friend in the biotechnology group Alhan, Wessam, and Mohammad Altib, who supported me morally in this foreign land and as my second family here in Malaysia. This also goes to Suha and Dalia for supporting me with lots of love and care.

I would like to extend my deepest gratitude to my everlasting friend Loai Abdelati Siddig who supports and stand with me during every moment in Malaysia and providing me the strength to face all my problems and to accomplish the study. I will always say my prayers to him.

I would like to dedicate this master's degree to my parents especially my mother, and hope that she is proud of me. I hope she is proud of me and I am most grateful for all the things she has done for me and hopefully I would be able to achieve my doctorate one day.

I am deeply indebted to my children Ftoon, Dalaa and Mohammed for being patient with me, while I complete this project.

Finally, I recognize that this master's degree would not be possible without the support from the greatest person in my life Hisham Badroon. I am most grateful for his advice in my life and his guidance for me to be able to finish this degree with his love and patience

Nassrin Abduljalel Badroon

February 2013

Abbreviation

× g	Acceleration due to gravity
®	Registered
°C	Degrees of centigrade
BHP	Benign hypertrophy of prostate
BPH	Benign prostate hyperplasia
bp	Base pair
C4-2	Androgen-hypersensitive cell line
CaCl ₂	Calcium chloride
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
dH ₂ O	Distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleic triphosphate
dTTP	Deoxythymidine triphosphate
EDTA	Enthylenediaminetetraacetic acid
<i>et al.</i>	et alii (and the rest)
etc	et cetera (and things)
g	gram
HCl	Hydrochloric acid
IPTG	Isopropythio-β-D-galactoside
kb	Kilobase
KoAC	Potassium acetate
KOH	Potassium hydroxide

LNCaP	Androgen-sensitive human prostate adenocarcinoma cells
LB	Luria-bertani
M	Moles
mg	Milligram
mg/ml	Milligram per milliliter
MgCl	Magnesium chloride
ml	Milliliter
ml	Milliliter
mm	Millimeter
mM	Micro molar
MOPS	3-(N-morpholino) propanesulfonic acid
N	Normality
NaCl	Sodium chloride
ng	Nano gram
nm	Nanometer
OD	Optical density
PC3/PC-3	Human prostate cancer cell lines
PCR	Polymerase chain reaction
<i>Pfu</i>	<i>Pyrococcus furiosus</i>
RbCl	Rubidium chloride
RNA	Ribonucleic acid
rpm	Revolution per minute
SDS	Sodium dodecyl sulphate
<i>Taq</i>	<i>Thermus aquaticus</i>
U	Unit

USA	United States of America
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactoside
$\mu\text{g/ml}$	Microgram per milliliter
μl	Micro liter
μM	Micro molar

List of figures	Page
Figure 1.1: Normal prostate anatomy	1
Figure 1.2: Prostate-specific membrane antigen (PSMA) structure	13
Figure 1.3: The process of overlap extension polymerase chain reaction	17
Figure 3.1: Two steps PCR for G1-d fragment of the <i>PSMA</i> gene.	38
Figure 3.2: Gel extraction for the G1-d fragment of the <i>PSMA</i> gene.	39
Figure 3.3: Two steps PCR for the construction of G1 fragment of the <i>PSMA</i> gene.	40
Figure 3.4: PCR amplification of the G2 fragment of the <i>PSMA</i> gene using <i>Pfu</i> polymerase.	41
Figure 3.5: PCR amplification of the G3 fragment of the <i>PSMA</i> gene using <i>Pfu</i> polymerase.	42
Figure 3.6: (a) <i>PSMA</i> gene fragments before gel extraction (b) <i>PSMA</i> gene fragments after gel extraction.	43
Figure 3.7: Overlap extension of G1 and G2 fragments.	44
Figure 3.8: A temperature gradient PCR for the amplification of G1-G2.	45
Figure 3.9: Serial dilution of template for G1-G2 PCR.	46
Figure 3.10: Gel extraction of amplified PCR product of G1, G2 and G3.	47
Figure 3.11: Colony PCR of PSMA G1, G2 and G3.	48
Figure 3.12: Plasmid Isolated for clones of PSMA G1, G2 & G3.	49
Figure 3.13: PCR product of amplifying PSMAG1, G2 &G3 from the diluted plasmid.	50
Figure 3.14: G1 sequencing results (a) showed the deletion of G nucleotide at 459 in G1 sequencing in A3 sample (b) showed the deletion of G nucleotide at 845 in G1 sequence in A5 sample.	51
Figure 3.15: Colony PCR amplifications using M13F and M13R primers.	52
Figure 3.16: Plasmid isolated from colonies with the right insert of G1.	52

Figure 3.17: PCR verification of the plasmid isolated from colonies of G1.	53
Figure 3.18: (a) Sequencing results of G1-a1 (b) G1- a2 and (c) G1-a4 and the figure showed the replaced nucleotide and the repairing area according to the amino acid codon.	54
Figure 3.19: Two steps PCR was done, 1st step was overlapping with <i>Pfu</i> polymerase enzyme & 2 nd step amplifying with <i>Pfu</i> polymerase enzyme.	55
Figure 3.20: Two steps PCR was done, 1 st step overlapping with <i>Pfu</i> polymerase enzyme and 2 nd step amplifying with <i>Taq</i> polymerase enzyme.	56
Figure 3.21: Gel extraction of PSMAG1-2, PSMAG2-3 & PSMAG1-2-3.	57
Figure 3.22: PCR amplification of G1 using plasmid (colony a4) as template with one unit of <i>Pfu</i> polymerase enzyme and one unit of <i>Taq</i> polymerase enzyme.	58
Figure 3.23: PCR amplification of G1 using plasmid (colony a4) as template with 1.5 unit of <i>Pfu</i> polymerase enzyme	58
Figure 3.24: (a) Overlap PCR of PSMA G2 and G3 fragments using one unit of <i>Taq</i> polymerase enzyme. (b): Overlap PCR of PSMA G2 and G3 fragments using two units of <i>Taq</i> polymerase enzyme.	59
Figure 3.25: Overlap PCR of PSMA G2 and G3 fragments. Amplify PCR done by using 2U of tag for each PCR reaction. (a) The PCR run in PCR machine (Peltier Thermal Cycler MJ Research). (b) The PCR run in PCR machine (PCR system 2400).	60
Figure 3.26: Overlap PCR of PSMA G2 and G3 fragments from different DNA templates.	61
Figure 3.27: Gel extraction of the PCR of G1 fragment from a4 colony plasmids.	62
Figure 3.28: Gel extraction of the PCR of G2-G3 fragment.	62
Figure 3.29: Overlap PCR of PSMA G2-G3 and PSMA G1.	63
Figure 3.30: PCR optimization of PSMA G1-G2-G3 sample through annealing temperature ranging between 45°C to 63.4 °C.	63

Figure 3.31: Gel extraction of the amplified PSMA G1-G2-G3.	64
Figure 3.32: Colony PCR of PSMA G1-2-3 by M13 primers forward and reverse.	65
Figure 3.33: Plasmid isolation of PSMA G1-2-3 of the sample A2 and A3.	65
Figure 3.34: Analysis of <i>Sfi</i> I and <i>Not</i> I restriction enzyme.	66
Figure 3.35: Colony PCR of PSMA G123.	67
Figure 3.36: Plasmid isolation of PSMA G123.	68
Figure 3.37: Linearization of PSMA G123 after digestion with <i>Sac</i> I.	68

List of table	Page
Table 1.1: TNM staging system of prostate cancer.	11
Table 2.1: Primer combination and the amplified products	25
Table 3.1: PSMA fragment combinations	44
Table 3.2: Template combination for overlapping PCR	55

Table of contents

Abstract	xi
Acknowledgement	xi
Abbreviations	ixi
List of figure	vii
List of table	x
1.0 Introduction	
1.1 Prostate gland	1
1.2 Prostate Diseases	2
1.3 Prostate Cancer	6
1.3.1 History of Prostate Cancer	6
1.3.2 Definition of Prostate Cancer	7
1.3.3 Genetics of cancer	8
1.3.4 Signs and symptoms	9
1.3.5 Screening and diagnosis	10
1.3.6 Prognosis	11
1.4 Prostate specific membrane antigen (PSMA)	12
1.4.1 Structure of PSMA	12
1.4.2 Dimerization of PSMA	14
1.4.3 Potential role of PSMA enzyme activity	15
1.4.4 Genomic properties	15
1.5 Polymerase Chain Reaction (PCR)	16
1.6 Overlap extension PCR	17
1.7 TA Cloning	18
1.8 Expression system	19
1.8.1 Bacterial expression system	19
1.8.2 <i>Pichia pastoris</i> expression system	20
1.9 Objectives	22
2. Materials & Methods	
2.1 Overlapping PCR	23
2.2 Gel electrophoresis	27
2.3 Gel Extraction	27
2.4 Cloning	27
2.4.1 Competent Cell Preparation	27
2.4.2 LB plates with ampicillin / IPTG / X-Gal	29
2.4.3 LB Broth Preparation	29
2.4.4 Ligation reaction with pGEM®-T vectors	29
2.4.5 Transformation	30
2.4.6 Recombinant Colonies Screening	31
2.5 Isolation of Plasmids	31
2.5.1 Plasmid digestion with <i>Sfi</i> 1 and <i>Not</i> 1	33
2.5.2 Plasmid purification	34
2.6 Cloning with pPICZαA vector	34
2.6.1 Ligation reaction with pPICZαA vector	34
2.6.2 Preparation of low salt Luria Bertani (LSLB) medium	34
2.6.3 Transformations into Top10 cells	35

2.6.4 Recombinant colonies screening	36
2.6.5 Colony PCR	36
2.7 Transformation into <i>Pichia pastoris</i>	36
3. Results	
3.1 Construction of <i>PSMA</i> gene from PCR product	37
3.1.1. Construction of G1	37
3.1.2 Amplification of PSMA G2	41
3.1.3 Amplification of PSMA G3	42
3.1.4 Purification of <i>PSMA</i> gene fragments	43
3.1.5 Overlap extension from PCR product	44
3.1.6 PCR optimization to amplify G1-G2 after overlapping	45
3.2. Cloning	47
3.2.1 Cloning of PSMA G1, G2 and G3	47
3.2.2 Sequencing result of PSMA G1, PSMA G2 and PSMA G3	51
3.2.3 Re-cloning of G1 Fragment	52
3.2.4 Overlapping of PSMA G1, G2 and G3 fragments from plasmid isolation product	55
3.2.5 Sequencing of the overlapped fragments	57
3.2.6 PCR optimization to amplify G1	58
3.2.7 PCR Optimization of overlapping G2-G3	59
3.2.8 Overlapping PSMA G2-G3 with G1	63
3.3 Plasmid digestion with <i>Sfi</i> 1 and <i>Not</i> 1	66
3.4 Colony PCR of PSMA G123	67
3.5 Linearization of PSMA G123	68
4. Discussion	69
5. Conclusion	77
Reference	78
Appendix	
(a) pGEM-T and pPICZ α A vectors features diagram	81
(b) Primers used for amplification of G1, G2 and G3 fragments of PSMA (overlapping extension).	82
(c) Full sequence of PSMA G1, G2 and G3 fragments	83