Abstract

In this study, *PSMA* was divided into 3 fragments, G1, G2 and G3. Two-step PCR was done to constructed 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 *Sfi1* and *Not1*. It was then purified and ligated into expression vector pPICZαA, which had been predigested with *sfiI* and *NotI*. Later on, it was transformed into Top10 competent cells. This recombinant construct (pPICZαA-PSMA) was isolated and linearized with restriction enzyme *Sac1*.

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_2O	Distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleic triphosphate
dTTP	Deoxythymidine triphosphate
EDTA	Enthylenediaminetetraacetic acid
et al.	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
Pfu	*Pyrococcus furiosus*
RbCl	Rubidium chloride
RNA	Ribonucleic acid
rpm	Revolution per minute
SDS	Sodium dodecyl sulphate
Taq	*Thermus aquaticus*
U	Unit

USA	United States of America
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
µg/ml	Microgram per milliliter
µl	Micro liter
µM	Micro molar

List of figures Page

List of table Page

Table of contents