# CONSTRUCTION OF FULL-LENGTH pKT9FL CLONE ENCODES FOR GLUTELIN FROM OIL PALM

LATISMA AETHAR

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU

2008

# CONSTRUCTION OF FULL-LENGTH pKT9FL CLONE ENCODES FOR GLUTELIN FROM OIL PALM

By
Latisya Binti Azhar

A research report submitted in partial fulfillment of
the requirements for the award of the degree of
Bachelor of Science (Biological Sciences)

**DEPARTMENT OF BIOLOGICAL SCIENCES**
**FAKULTI OF SCIENCE AND TECHNOLOGY**
**UNIVERSITI MALAYSIA TERENGGANU**
**2008**

This project should be cited as:

Latisya, A. Construction of Full-Length pKT9FL Clone Encodes for Glutelin from Oil Palm. Undergraduate thesis, Bachelor of Science (Biological Sciences), Faculty of Science and Technology, University Malaysia Terengganu. 48pp.

**JABATAN SAINS BIOLOGI**
**FAKULTI SAINS DAN TEKNOLOGI**
**UNIVERSITI MALAYSIA TERENGGANU**

## PENGAKUAN DAN PENGESAHAN LAPORAN
## PROJEK PENYELIDIKAN I DAN II
## *RESEARCH REPORT VERIFICATION*

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **CONSTRUCTION OF FULL-LENGTH pKT9FL CLONE ENCODES FOR GLUTELIN FROM OIL PALM** *o*leh **LATISYA BINTI AZHAR** no. matrik: **UK11245** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah **SARJANA MUDA SAINS (SAINS BIOLOGI)**., Fakulti Sains dan Teknologi,Universiti Malaysia Terengganu.

Disahkan oleh: /Verified by:

.....

Penyelia Utama/*Main Supervisor*
Nama: **DR. CHA THYE SAN**
Cop Rasmi: **DR. CHA THYE SAN**
Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh: 4/5/2008

.....

Ketua Jabatan Sains Biologi/*Head, Department of Biological Sciences*
Nama: **PROF. MADYA DR. AZIZ BIN AHMAD**
Cop Rasmi:
PROF. MADYA DR. AZIZ BIN AHMAD
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 04 MAY 2008

## DECLARATION

I hereby declare that this thesis entitled Construction of Full-Length pKT9FL Clone Encodes for Glutelin from Oil Palm is the result of my own research except as cited in the references.

Signature 
Name : LATISYA BT AZHAR
Matrix No : UK11245
Date : 30th April 2008

## ACKNOWLEDGEMENT

Praise to Allah for His Will in giving me the strength to complete this research which fulfilled my degree requirement.

I would like to express my utmost gratitude to my supervisor Dr. Cha Thye San for his guidance and encouraged me throughout this project. His scientific excitement inspired and it built my enthusiasm for me to involve in this molecular biology field. All the discussions made during this project really enrich my knowledge especially in molecular biology and experienced gained in the lab was really a precious moment for me. The knowledge and expertise Dr. Cha shared with me will undoubtedly useful for me in the future undertakings. Without his assistance and support I would not been able to complete my degree.

Not forgotten, I would like to give my warm thanks to all my friends under supervision of Dr. Cha for their support and encouragement especially those under the Dr. Cha Thye San supervision for that helps me throughout my final year project. All the good times and laughs kept me afloat when times were tough. I also like to thank master student for their advice, guidance, and sharing their knowledge with me in molecular biology throughout accomplishing this project.

Finally, I would like to acknowledge all lab assistant especially Madam Normaiziie for her helps in providing me the material I need to used during my project. It has been privilege and pleasure for me to have the chance to work and use the facilities in Biotechnology lab, Molecular Biology lab and also Microbiology lab provided by University Malaysia Terengganu.

Last but not least, I would like to send my warmest thanks to my family for always standing by my side, encourage and believe in me through all this years.

This project might not be successfully developed without the support and assistance of many people. Thank you again to everyone who involved in accomplishing this project.

## ABSTRACT

Seed storage protein is classified into three classes, which are globulin, prolamin and glutelin. The protein was utilized during post-germinative period of plant growth. Three different isoforms of glutelin genes were isolated from oil palm kernel (*Elais guineesis*, Tenera) in previous research. One of the isoforms consists of two separate fragments, the 3'-end-fragment (900 bp) isolated from cDNA library and the 5'-end fragment (820 bp) isolated by using 5'-RACE method. The full length of this clone is 1621 bp. Thus, this study was aimed to construct a complete full-length sequence of this oil palm glutelin cDNA by joining the two fragments at the overlapping sequence. The 5'-end fragment was first cloned into *Eco*Rl and *Sma*l site of pUC19 vector. The 3'-end fragment was generated by PCR and joined with the 5'-end fragment at the *Hpa*l site located at nt. 894 of the glutelin gene. The positive recombinant clone was selected by colony PCR technique and named as pKT9FL. The orientation and the joining site of *Hpa*l in clone pKT9FL were verified by DNA sequencing. This complete sequence of clone pKT9FL consists of 1621 bp that encodes a polypeptide of 469 amino acid. Its 5'and 3'-untranslated region is 52 bp and 152 bp respectively.

## ABSTRAK

Protein simpanan biji di kelasifikasikan kepada tiga kelas iaitu globulin, prolamin dan glutelin. Protein simpanan biji digunakan ketika fasa selepas percambahan dalam kitaran perkembangan tumbuhan. Dalam kajian terdahulu, tiga isoform gen glutelin yang berbeza telah dipencilkan daripada isirong kelapa sawit (*Elais guineesis*, Tenera). Salah satu isoform, terdiri daripada dua serpihan yang berasingan iaitu bahagian 3'-akhiran (900bp) yang dipencilkan daripada perpustakaan cDNA isirong manakala bahagian 5'-akhiran dipencil daripada eksperimen 5'-RACE. Saiz lengkap jujukan klon itu ialah 1621 bp. Oleh itu, objektif utama penyelidikan ini adalah untuk membentuk jujukan lengkap klon cDNA gen glutelin kelapa sawit ini dengan menyambungkan kedua-dua serpihan pada kawasan bertindih. Bahagian 5'-akhiran diklonkan pada tapak *Eco*RI dan *Sma*I pada vektor pUC19. Manakala, bahagian 3'-akhiran pula diamplifikasi dengan PCR dan disambungkan pada bahagian 5'-akhiran pada tapak *Hpa*I (nt. 894) dalam vektor pUC19. Klon rekombinan positif dipilih melalui teknik PCR koloni dan dinamakan pKT9FL. Orentasi jujukan dan tapak penyambungan *Hpa*I dalam klon pKT9FL telah dipastikan melalui penjujukan DNA. Jujukan lengkap klon ini ialah 1621 bp yang mengekodkan polipeptida sepanjang 469 amino asid. Saiz kawasan 5' dan 3' yang tidak mengekod ialah masing-masing 52 bp dan 152 bp.