

การพัฒนาชุดจำแนกพันธุ์กรรมในกึ่งฤดูดำ *Penaeus monodon* Fabricius
โดยเทคนิคไมโครแซเทลไลต์



นายพิทักษ์ สุตรอนันต์

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาชีวเคมี ภาควิชาชีวเคมี

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2542

ISBN 974-334-203-6

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

DEVELOPMENT OF DNA TYPING KIT IN BLACK TIGER PRAWN

***Penaeus monodon* Fabricius BY MICROSATELLITE TECHNIQUE**



Mr. Pitak Soot-anan

**สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย**
**A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Biochemistry**

Department of Biochemistry

Faculty of Science


Chulalongkorn University

Academic Year 1999

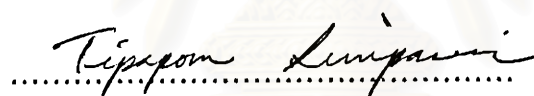
ISBN 974-334-203-6


Thesis Title Development of DNA typing kit in black tiger prawn *Penaeus monodon* Fabricius by microsatellite technique
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Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master's Degree.

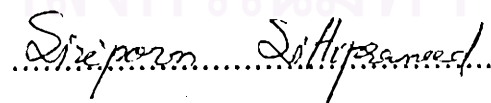

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พิทักษ์ สุตรอนันต์ : การพัฒนาชุดจำแนกพันธุกรรมในกุ้งกุลาดำ *Penaeus monodon* Fabricius โดยเทคนิคไมโครแซเทลไลต์ (DEVELOPMENT OF DNATYPING KIT IN BLACK TIGER PRAWN *Penaeus monodon* Fabricius BY MICROSATELLITE TECHNIQUE) อ. ที่ปรึกษา : รศ. ดร. อัญชลี ทศนาขจร, อ. ที่ปรึกษาร่วม : ดร. ศิราวุธ กลิ่นบุหงา, 116 หน้า. ISBN 974-334-203-6

ทำการคัดเลือกไมโครแซเทลไลต์จำนวน 8 ตำแหน่ง ได้แก่ CSCUPmo1, CSCUPmo2, CSCUPmo3, CSCUPmo4, CSCUPmo6, CSCUPmo7, CSCUPmo9 และ CSCUPmo11 เพื่อใช้ในการจำแนกพันธุกรรมกุ้งกุลาดำ โดยศึกษาความหลากหลายในกลุ่มประชากรกุ้งจากจังหวัดตราดประมาณ 50 ตัว พบจำนวนอัลลีลของแต่ละตำแหน่งเท่ากับ 29, 27, 27, 21, 29, 22, 33 และ 10 อัลลีล ตามลำดับ ได้ค่าเฮเทอโรไซโกซิติ (heterozygosity) ในช่วง 0.21 ถึง 0.90 และให้ขนาดของอัลลีลตั้งแต่ 132 ถึง 380 คู่เบส คัดเลือกเฉพาะเครื่องหมายไมโครแซเทลไลต์ 6 ตำแหน่งที่ให้ผลความหลากหลายสูง และให้แถบดีเอ็นเอที่ชัดเจน เพื่อนำมาใช้ในการตรวจสอบยีนโตนิกวินไมโครแซเทลไลต์ที่ตำแหน่ง CSCUPmo3 กับ CSCUPmo7 ที่อ่านผลยาก และ ให้ยีนโตนิกวินแบบโฮโมไซกัส (homozygous) มากผิดปกติ ชุดจำแนกพันธุกรรมของกุ้งกุลาดำโดยเทคนิคไมโครแซเทลไลต์ ที่ได้รับการพัฒนา ต้องการตัวอย่างเนื้อเยื่อ หรือ เลือดในเอทานอล เพียง 10 มิลลิกรัม หรือ 5 ไมโครลิตร ตามลำดับ สำหรับการสกัดดีเอ็นเอด้วยวิธีอัลคาไลน์ การพัฒนาวิธีการวิเคราะห์ไมโครแซเทลไลต์แบบมัลติเพลกซ์ (multiplex) ช่วยให้สามารถตรวจสอบยีนโตนิกวินของกุ้งได้พร้อมกันที่ละหลายตำแหน่ง ซึ่งพบว่าไมโครแซเทลไลต์ที่ตำแหน่ง CSCUPmo1 และ CSCUPmo2 สามารถทำมัลติเพลกซ์ที่ซิวอาร์ได้ ส่วนคู่ไมโครแซเทลไลต์ที่ตำแหน่ง CSCUPmo4 และ CSCUPmo9 กับคู่ไมโครแซเทลไลต์ที่ตำแหน่ง CSCUPmo6 และ CSCUPmo11 มีขนาดของผลิตภัณฑ์ซิวอาร์ที่ไม่ซ้อนกัน สามารถนำมาวิเคราะห์โดยหยอดตัวอย่างพร้อมกันได้ นอกจากนี้สามารถตรวจสอบขนาดของไมโครแซเทลไลต์อัลลีลโดยไม่ใช้สารรังสี โดยนำผลิตภัณฑ์ซิวอาร์ที่ได้มาแยกขนาดด้วย 8% denaturing polyacrylamide sequencing gel และนำมาข้อมด้วยซิลเวอร์ (silver staining) ส่วนไมโครแซเทลไลต์ที่มีขนาดของอัลลีลต่างกันตั้งแต่ 3 เบส ขึ้นไป ได้แก่ ไมโครแซเทลไลต์ที่ตำแหน่ง CSCUPmo11 สามารถแยกความแตกต่างของอัลลีลโดยใช้ 15% denaturing polyacrylamide minigel ซึ่งสะดวก และรวดเร็วกว่าการใช้ sequencing gel และสามารถสร้างดีเอ็นเอมาตรฐานสำหรับจำแนกขนาดของอัลลีลที่ตำแหน่ง CSCUPmo11 ได้ ซึ่งช่วยให้การอ่านยีนโตนิกวินง่ายและถูกต้องมากยิ่งขึ้น

ภาควิชา ชีวเคมี
สาขาวิชา ชีวเคมี
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ลายมือชื่อนิสิต
ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

4072334623 : MAJOR BIOCHEMISTRY

KEY WORD: *Penaeus monodon* / black tiger prawn / microsatellite / DNA typing kit

PITAK SOOT-ANAN : DEVELOPMENT OF DNA TYPING KIT FOR BLACK TIGER PRAWN *Penaeus monodon* Fabricius BY MICROSATELLITE TECHNIQUE. THESIS ADVISOR : ASSOC. PROF. ANCHALEE TASSANAKAJON, Ph.D. THESIS CO-ADVISOR : SIRAWUT KLINBUNGA, Ph.D. 116 pp. ISBN 974-334-203-6

Eight microsatellite loci, CSCUPmo1, CSCUPmo2, CSCUPmo3, CSCUPmo4, CSCUPmo6, CSCUPmo7, CSCUPmo9 and CSCUPmo11 were investigated to select suitable microsatellite loci for use in DNA typing of the black tiger prawn *Penaeus monodon*. Allelic variations were examined in 50 individuals *P. monodon* from Trad. All eight loci were be highly polymorphic which exhibited number of alleles at each locus of 29, 27, 27, 21, 29, 22, 33 and 10 alleles, respectively and heterozygosities between 0.21-0.90. Size ranges of alleles varied from 132-380 bp. Six microsatellite loci were chosen based on the level of polymorphism and the ease of allele scoring. The CSCUPmo3 and CSCUPmo7 loci that gave ambiguous allelic patterns and an excess of homozygous genotypes were discarded from further analysis. Approximately 10 mg and 5 μ l of the tissues and blood/ethanol respectively were using for DNA typing of *P. monodon* by microsatellite technique. Alkaline extraction method was the most simple and appropriate DNA isolation when dealing with a large number of specimens. Multiplex analysis was developed to provide rapid amplification of multiple loci simultaneously. Successful analyses were multiplex PCR of loci CSCUPmo1 and CSCUPmo2 and single loading of combined amplified products of paired microsatellites (CSCUPmo4+CSCUPmo9, CSCUPmo6+ CSCUPmo11). Non-isotopic method were used to detect amplified alleles by separating in 8% denaturing polyacrylamide sequencing gels and visualization of amplified alleles using silver staining. Allele difference of 3 bases such as those of the locus CSCUPmo11 could also be detected by using 15% denaturing polyacrylamide minigel. This allowed simpler and more rapid detection of microsatellite alleles. Construction of allelic ladders for use as standard DNA markers was succeeded for the locus CSCUPmo11. The allelic ladders make band scoring simple and more precise.

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ลายมือชื่อนิสิต
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

Acknowledgments

I would like to express my deepest gratitude to my advisor, Associate Professor Dr. Anchalee Tassanakajon, for her valuable guidance, correcting grammatical errors and encouragement with her kindness mind throughout this study. I am very grateful to my co-advisor, Dr. Sirawut Klinbunga for his great help, guidances, and suggestions in my thesis. The special thanks are also extended to Dr. Padermsak Jarayabhand for his help in supporting the samples.

Special thanks are given to Miss Siriporn Pongsomboon for her primer development and Miss Premruithai Supungul for technical guidance and assistance. My gratitude is also extended to all of my friends, and all staff members of the Department of Biochemistry, Faculty of Science, Chulalongkorn University for their helps and friendship during my study. Much appreciation is expressed to all teachers and friends in RCU. Mr. Phao Suwannasaksri for sincerity help. My special appreciation is also expressed to my roommate Mr. Suparak, Mr. Therawat for friendship and Mr. Pongrat for using his computer and friendship. I would like to express my deepest appreciation to Miss Busara Sripanit for her friendship.

I wish to acknowledge to contributions of the Thailand Graduate Institute of Science and Technology, TGIST, National Science and Technology Development Agency, NSTDA).

Finally, I wish to express extremely grateful for the constant encouragement received from my parents, my sister and my brothers for giving me the warmest life with their love.

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LIST OF ABBREVIATIONS

bp	Base pair
°C	Degree celsius
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
dTTP	Deoxythymidine triphosphate
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetic acid (disodium salt)
H	Mean heterozygosities
h	heterozygosity
HCl	Hydrochloric acid
kb	Kilobase pair (10^3 bp)
MgCl ₂	Magnesium choride
min	Minute
ml	Millilitre (10^{-3} litre)
mM	Millimolar
MT	Metric tonnes
mtDNA	Mitochondrial DNA
ng	Nanogram (10^{-9} gram)
OD	Optical density
PCR	Polymerase chain reaction
Rnase	Ribonuclease
SDS	Sodium dodecyl sulfete
Sec	Second

T_A	Annealing temperature
TE	Tris EDTA
Tris	Tris (hydroxy methyl) aminomethane
V	Volt
VNTR	Variable number of tandem repeats
W	watt
μ l	Microlitre (10^{-6} litre)



สถาบันวิทยบริการ
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