

IDENTIFICATION OF MICRORNA GENES AND THEIR TARGETS IN
WHITELEG SHRIMP (*Litopenaeus vannamei*)

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THIS DISSERTATION IS PRESENTED AS PARTIAL FULFILLMENT FOR THE
REQUIREMENTS TO OBTAIN A MASTER OF BIOTECHNOLOGY

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
2009

DECLARATION

I hereby declare that this thesis is my original work, except for some quotations and references that have been dully acknowledged.

9 March 2009

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SGF070018

ACKNOWLEDEMENTS

First and foremost, I would like to take this opportunity to express my heartfelt gratitude to both my supervisors, Associate Professor Dr. Jennifer Ann Harikrishna and Dr. Subha A/P Bhassu for their guidance, suggestions and encouragements throughout this research.

Furthermore, I would like to thank and be grateful to my parents for being so supportive and tolerance. In addition, a highly appreciation is also dedicated to Dr. Subha A/P Bhassu for her willingness to supply her lab, consumable chemicals and equipments in my study. Besides that, special thanks are bestowed to University of Malaya for approving RM10K grant for research use in this study.

Hereby, my heartfelt thank should also go to all my lab colleagues for their endless help and supports. Last but not least, I would like to express my sincere appreciation to the staffs of Institute of Biological Science for their help in my research.

ABSTRAK

PENGENALAN GEN-GEN MIKRORNA DAN SASARAN MEREKA DALAM UDANG PUTIH (*Litopenaeus vannamei*)

Gene mikroRNA merupakan pengawal yang penting dalam gen ekspresi eukaryotik. Banyak gen mikroRNA telah dikenal pasti berada dalam kebanyakan organisma. Tetapi, bagi kehidupan air, gen-gen mikroRNA hanya dikaji terhadap ikan bertulang sejati jenis *Danio rerio*, jenis *Fugu rubripes* dan jenis *Tetraodon nigroviridis*. Oleh itu, pengajian tentang gen mikroRNA dalam kehidupan air yang lain memang diperlukan. Dengan kewujudan *Litopenaeus vannamei* EST pangkalan data dan kepentingannya dalam pasaran di akuakultur industri, udang putih (*Litopenaeus vannamei*) telah dipilih sebagai sasaran dalam penyelidikan ini. Dengan menggunakan rangkain gen-gen mikroRNA yang diketahui, kaedah berdasarkan homologi telah diaplikasikan untuk mengenalpastikan calon-calon mikroRNA daripada EST pangkalan data *Litopenaeus vannamei*. Sebanyak 11 calon-calon mikroRNA telah dikenal pasti melalui bioinformatik. Tetapi, hanya calon lva-miR-256, lva-miR-272 and lva-miR-1476-3p dipilih untuk eksperimen seterusnya dan kewujudan mereka disahkan daripada 6 jenis organ udang putih. Selain itu, miRNA: mRNA sasaran juga dikenalpastikan melalui bioinformatik. Terdapat 20 mRNA and 27 EST rangkaian telah diramalkan untuk menjadi sasaran bagi 11 calon-calon mmikroRNA. Dengan itu, fungsi-fungsi mikroRNA juga dapat diramalkan. Kebanyakan sasaran mikroRNA terlibat dalam pangawalan system biology seperti protein sistem pertahanan, enzim-enzim, faktor-faktor pentranscriptan, kematian sel, proses metabolik dan proses pembangunan. Lva-miR-256 diramalkan mengawal proses pembinaan, metabolik dan sistem sel; lva-miR-272 pula, diramalkan terlibat dalam sistem sel dan lva-miR-1476-3p diramal untuk mengawal proses pembinaan dan sistem sel.

ABSTRACT

MicroRNA genes are the vital regulators in eukaryotic gene expression. MicroRNA genes have been identified in various organisms. However, in aquatic animals, miRNA genes have been only annotated in zebrafish (*Danio rerio*) and puffer fish (*Fugu rubripes* and *Tetraodon nigroviridis*). Therefore, there is a need to study on miRNA genes in other aquatic animals. Due to availability of *Litopenaeus vannamei* EST database and its important market value in aquaculture industry, whiteleg shrimp (*Litopenaeus vannamei*) was selected in this study. Using known miRNA gene sequences, homology-based searching approach was employed to identify miRNA candidates in *Litopenaeus vannamei* EST database. 11 miRNA candidates were computationally identified. However, Lva-miR-256, lva-miR-272 and lva-miR-1476-3p were selected to be experimentally validated in 6 different target tissues. Apart from that, miRNA: mRNA target search has also been performed. 20 mRNA and 27 EST sequences were predicted to be targeted by 11 miRNA candidates. Therefore, the function of miRNA candidates could be predicted. These miRNA candidates are found to regulate wide range of functions, including defense-related proteins, enzymes, transcription factors, cell death, metabolic and developmental process. Lva-miR-256 is most likely involved in cellular, developmental and metabolic processes; lva-miR-272 potential regulates cellular processes and lva-miR-1476-3p regulates mostly cellular and developmental processes.

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LIST OF SYMBOLS

bp	Base pairs
°C	Degree Celsius
%	Percentage
μl	Microliter
μg	Microgram
M	Molar
mM	Milimolar
ml	Mililiter
g	Gravity
V	Voltage

LIST OF ABBREVIATION

ame-miR	<i>Anopheles gambiae</i> MicroRNA
bmo-miR	<i>Bombxy mori</i> MicroRNA
cel-miR	<i>Caenorhabditis elegans</i> MicroRNA
cin-miR	<i>Ciona intestinalis</i> MicroRNA
cds	coding sequences
dbRNA	double stranded RNA
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleoside Triphosphates
EtBr	Ethidium Bromide
lva-miR	<i>Litopenaeus vannamei</i> MicroRNA
miRNA	MicroRNA
mRNA	Messenger RNA
NCBI	National Centre for Biotechnology Information
NGS	Next generation sequencing
ncRNAs	Non-coding Ribonucleic Acids
odi-miR	<i>Oikopleura dioica</i> MicroRNA
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
sRNA	Small Ribonucleic Acid
siRNA	Short Interference RNA
sme-miR	<i>Schmidtea mediterranea</i> MicroRNA
RNAi	RNA Interference
TBE	Tris-Borate-EDTA
3' UTR	3' Untranslated Region