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
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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
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ABSTRAK

PENGENALAN GEN-GEN MIKORNA DAN SASARAN MEREKA DALAM UDANG PUTIH (Litopenaues vannamei)

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