

**DEVELOPMENT AND ANALYSES OF EXPRESSED SEQUENCE TAGS
FROM *Gracilaria changii* FOR FUNCTIONAL GENOMIC STUDIES**

By

TEO SWEE SEN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Chairman : Ho Chai Ling, Ph.D.

Faculty : Biotechnology and Biomolecular Sciences

Macroalgae from the genus *Gracilaria* is the most common agarophytic genus in Malaysia (Phang *et al.*, 1996). This wild population of seaweed has been identified as an important source of raw material for the agar industry. Despite its potential to produce good gel strength agar, *Gracilaria* sp. was genetically less studied. The aims of this study are to generate and sequence a thousand Expressed Sequence Tag (EST) sequences from *G. changii* for further cDNA microarray to facilitate functional genomic research. RNA extraction from *G. changii* is difficult due to poor yield, polysaccharide contamination and gel formation. To circumvent these problems the RNA isolation procedure was modified and repeated more than 150 times (more than 10 kg of fresh samples were used) to obtain high quality RNA for further studies. From the three modified RNA extraction methods, the modified method of Kim *et al.* (1997) was chosen for rapid RNA isolation from *G. changii*. This method can be completed within 1 day and many samples can be processed at the same

time. The yield was increased from 0.018 $\mu\text{g/g}$ to 1.14 $\mu\text{g/g}$ of tissue with an average purity measured as $A_{(260/280)}$ of 1.90. After the modification, the mRNA was recovered from the total RNA of *G. changii* at a ratio of 0.5 – 1.0%. Starting from 5 μg of mRNA, a primary cDNA library of 1.14×10^6 clones was constructed and 1.375×10^{10} pfu/mL plaques were established for the amplified library. A total of 1854 cDNA clones were successfully sequenced. The database consists of ESTs with putative functions in protein synthesis (6%), energy (4%), protein destination and storage (3%), metabolism (3%), transportation (2%), transcription (2%), signal transduction (1%), cell structure/maintenance (1%), disease and defence (1%), cell growth and division (1%), intracellular traffic (1%) and other miscellaneous functions (2%). Putative proteins with unknown functions (67%), and novel sequences (6%) that do not show significant matches to the existing sequence databases are also present. Among the ESTs, 1342 sequences (72.38%) were clustered as singleton, and the remaining 512 were clustered into 168 contigs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMBANGUNAN DAN ANALISIS EXPRESSED SEQUENCE TAGS BAGI
Gracilaria changii UNTUK KAJIAN GENOMIK FUNGSIAN**

Oleh

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Genus *Gracilaria* merupakan makroalga penghasil agar yang paling umum di Malaysia (Phang *et al.*, 1996). Populasi liar rumpai laut ini telah pasti dikenal sebagai sumber bahan mentah penting untuk industri agar. Walaupun ia berpotensi dalam penghasilan agar dengan kekentalan gel yang baik, *Gracilaria* sp. kurang dikaji dari segi genetiknya. Tujuan kajian ini adalah untuk menghasilkan dan menjujukan seribu jujukan EST (Expressed Sequence Tag) dari *G. changii* untuk pengaturan mikro cDNA bagi memudahkan pengajian genomik fungsian. Ekstraksi RNA dari *G. changii* adalah sukar disebabkan penghasilan yang tidak memuaskan, pencemaran polisakarida dan pembentukan gel. Untuk mengelakan masalah-masalah ini, kaedah pemencilan RNA telah diubahsuaikan dan diulang lebih daripada 150 kali (lebih daripada 10 kg sampel segar telah digunakan) untuk memperolehi RNA yang berkualiti tinggi bagi pengajian seterusnya. Daripada tiga kaedah ekstraksi RNA yang telah diubahsuaikan, kaedah Kim *et al.* (1997) yang telah

diubahsuai telah dipilih sebagai kaedah pemencilan RNA daripada *G. changii*. Kaedah ini membolehkan banyak sampel diproses pada masa yang sama dan dapat ditamatkan dalam masa sehari sahaja. Penghasilan RNA telah ditingkatkan daripada 0.018 $\mu\text{g/g}$ kepada 1.14 $\mu\text{g/g}$ tisu dengan ketulenannya ($A_{(260/280)}$) 1.90. Setelah pengubahsuaian, nisbah mRNA yang diperolehi daripada jumlah RNA *G. changii* adalah 0.5 – 1.0%. Bermula dengan 0.5 μg mRNA, suatu khazanah cDNA yang mengandungi 1.14×10^6 klon telah dihasilkan. Khazanah cDNA yang telah diampifikasi mempunyai 1.375×10^{10} *pfu/mL*. Sejumlah 1854 EST telah berjaya diujukan. Pengkalan data yang terdiri daripada EST mempunyai fungsi putatif dalam sintesis protein (6%), tenaga (4%), penyimpanan dan destinasi protein (3%), metabolisme (3%), pengangkutan (2%), transkripsi (2%), transduksi isyarat (1%), struktur dan penyelenggaraan sel (1%), penyakit dan pertahanan (1%), pertumbuhan dan pembahagian sel (1%), intraselular trafik (1%) dan lain-lain kepelbagaian fungsi (2%). Protein putatif dengan fungsi yang tidak diketahui (67%) dan jujukan baru (6%) yang tidak menunjukkan persamaan yang signifikan kepada jujukan yang sedia ada pada pengkalan data juga dikesan. Di kalangan EST yang diperolehi, 1342 jujukan (72.38%) dikelompokkan sebagai 'singleton' dan yang selebihnya, 512 jujukan, dikelompokkan kepada 168 'contigs'.

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I certify that an Examination Committee met on date of viva to conduct the final examination of Teo Swee Sen on her degree in Master of Science thesis entitled “Functional Genomic Studies of *Gracilaria changii* (Gracilariales, Rhodophyta) ~ An Expressed Sequence Tag (EST) Approach” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

TEO SWEE SEN

Date:

3.5.1	Isolation of Poly ⁺ A RNA using μ MACS mRNA Isolation Kit	45
3.5.2	First-strand cDNA Synthesis	45
3.5.3	Second-strand cDNA Synthesis	46
3.5.4	Blunting the cDNA Termini	46
3.5.5	Ligating the <i>Eco</i> RI Adapters	47
3.5.6	Phosphorylating the <i>Eco</i> RI Sites	47
3.5.7	Digesting with <i>Xho</i> I	48
3.5.8	Size Fractionation by using QIAquick Gel Extraction Kit (Qiagen)	48
3.5.9	Ligation of cDNA into the Uni-ZAP XR Vector	49
3.5.10	Packaging Protocol for the Gigapack III Gold Packaging Extract	49
3.5.11	Preparation of Host Bacteria	49
3.5.12	Plating and Titering	50
3.5.13	Amplification of the cDNA Library	50
3.6	Identification of Insert	51
3.6.1	<i>In vivo</i> Excision	51
3.6.2	Mass Excision	52
3.6.3	Plasmid Preparation	53
3.7	PCR Amplification	54
3.8	DNA sequencing	54
3.9	Data Analysis	55
4.	RESULTS	57
4.1	Optimization of RNA Isolation from <i>G. changii</i>	57
4.2	mRNA Isolation	65
4.3	Construction of cDNA library	65
4.4	Random Screen Approach	68
4.4.1	<i>In Vivo</i> and Mass Excision	68
4.4.2	Plasmid Isolation	69
4.4.3	Polymerase Chain Reaction (PCR)	69
4.5	Expressed Sequence Tag (EST) Generation	73
4.5.1	ESTs Classification	80
5.	DISCUSSION	87
5.1	RNA Isolation from <i>G. changii</i>	87
5.2	mRNA Isolation	93
5.3	Construction of cDNA Library and Polymerase Chain Reaction (PCR) Amplification	93
5.4	Sequence Analysis of the <i>G. changii</i> ESTs	94
5.5	Functional Classification of <i>G. changii</i> ESTs	97
5.5.1	Protein Synthesis	101
5.5.2	Energy	101
5.5.3	Metabolism	102

5.5.4	Protein Destination and Storage	103
5.5.5	Transcription	104
5.5.6	Signal Transduction	104
5.5.7	Transporter	105
5.5.8	Disease and Defense	106
5.5.9	Cell Structure and Maintenance	106
5.5.10	Intracellular Traffic	107
5.5.11	Cell Growth and Division	107
5.5.12	Miscellaneous	108
6	CONCLUSION	109
	Future Work	110
	BIBLIOGRAPHY	112
	APPENDICES	119
	BIODATA OF THE AUTHOR	162