THE USE OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *CRASSOSTREA IREDALEI* (OYSTER) IN PCR AMPLIFICATION STUDY

KONG HUI JIE

FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
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THE USE OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *CRASSOSTREA IREDALEI* (OYSTER) IN PCR AMPLIFICATION STUDY

By

Kong Hui Jie

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Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
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FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: THE USE OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF SPECIES *CRASSOSTREA IREDALEI* (TIRAM) IN PCR AMPLIFICATION STUDY oleh Kong Hui Jie, no. matrik: uk 7815 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, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

.....

Penyelia Utama

Nama: WAN BAYANI WAN OMAR

Cop Rasmi: Jabatan Sains Biologi
Fakulti Sains Dan Teknologi
Kolej Universiti Sains Dan Teknologi Malaysia
Mengabang Telipot,
21030 Kuala Terengganu,
Terengganu.

Tarikh: 11/5/2006

.....

Penyelia Kedua (jika ada)

Nama:

Cop Rasmi

Dr. Zaleha Binti Kassim
Pensyarah
Jabatan Sains Samudera
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu

Tarikh: .....

.....

Ketua Jabatan Sains Biologi

Nama: PROF. MADYA DR. NAKISAH BT. MAT AMIN

Cop Rasmi: Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: .....

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°C	Degree Celsius
λ	Lambda
%	Percentage
bp	base pair
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediamine tetra-acetic acid
g	Gram
kb	Kilobase
L	Litre
µL	Microlitre
µg	Microgram
mL	Mililitre
mM	Milimolar
OD	Optical Density
rpm	Revolution per minute
SDS	Sodium Dodecyl Sulphate
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
TNES-urea buffer	Tris-NaCl-EDTA-SDS-urea buffer
v/v	volume/volume
w/v	weight/volume

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ABSTRACT

Crassostrea iredalei has been known as an important commercial species and have potential for aquaculture. The purity and quality of DNA extracted from tissue samples is important for sensitivity and usefulness of molecular methods such as RAPD-PCR. Therefore, the availability of effective DNA extraction methods is essential. Successful preservation of tissue sample is required for long term molecular studies in distant areas to prevent DNA degradation. In this study, the best preservative and DNA extraction method that produce DNA of highest purity and quality was determined. Two different preservatives were used to preserve the tissue samples and two different DNA extraction methods were used to extract the genomic DNA for PCR amplification. Fresh tissues were used as a control. The purity and quantity of extracted DNA was measured with a spectrophotometer and verified by agarose gel electrophoresis. Finally, the extracted DNA was selected for RAPD-PCR. The purity and quantity of DNA extracted from 95% ethanol was ranged from 1.078 to 1.291 and 260.0 ng/μL to 492.5 ng/μL .The DNA purity and quantity of DNA extracted from TNES-urea buffer was in range of 1.167 to 1.355 and 302.5 ng/μL to 505.0 ng/μL respectively. Based on the banding patterns generated by agarose gel electrophoresis, the Promega Wizard™ Genomic DNA Purification Kit was a good DNA extraction method compared to Phenol-chloroform method and the TNES-urea buffer preservative is a good preservative for *Crassostrea iredalei*.

PENGGUNAAN DUA BAHAN AWET BERLAINAN DAN KAEDAH PENGEKSTRAKAN DNA BERLAINAN BAGI TISU *CRASSOSTREA IREDALEI* (TIRAM) DALAM KAJIAN AMPLIFIKASI PCR

ABSTRACT

Crassostrea iredalei adalah spesies komersial yang penting dan mempunyai potensi untuk akuakultur. Ketulenan DNA yang diekstrak daripada tisu sampel adalah penting bagi kepekaan kaedah molekular seperti RAPD-PCR dalam pemilihan stren *C. iredalei* yang baik. Pengawetan tisu yang baik diperlukan untuk kajian molekular di kawasan yang jauh untuk mengelakkan degredasi DNA. Dalam kajian ini bahan awet dan keadah pengekstrakan DNA yang menghasilkan DNA yang paling tulen dan berkualiti ditentukan. Dua kaedah pengawetan yang berbeza digunakan untuk mengawet tisu dan dua kaedah pemencilan DNA digunakan untuk memencilkan DNA genomik untuk amplifikasi PCR. Ketulenan dan kuantiti DNA ditentukan dengan spektrofotometer dan diverifikasi dengan elektroforesis gel agaros. Akhirnya, DNA yang diekstrak dipilih untuk RAPD-PCR. Ketulenan dan kuantiti DNA yang dipencil daripada tisu dalam pengawet 95% ethanol adalah dalam julat 1.078 hingga 1.291 dan 260 ng/μL hingga 492.5 ng/μL masing-masing, manakala daripada tisu dalam pengawet TNES-urea buffer adalah dalam julat 1.167 hingga 1.355 dan 302.5 ng/μL hingga 505.0 ng/μL. Keputusan berdasarkan corak jaluran elektroforesis menunjukkan kaedah ekstraksi DNA yang paling efisyen adalah Wizard™ Genomic DNA Purification Kit dari Promega manakala penimbal TNES-urea adalah bahan pengawet yang sesuai bagi *Crassostrea iredalei*.