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Different preservatives and dna extraction methods of Polymesoda Expansa (clam) in pcr amplification study / Norasma Zakaria.

Lihat sebelah

DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS OF *POLYMESODA EXPANSA* (CLAM) TISSUES IN PCR AMPLIFICATION STUDY

By

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Research Report submitted in partial fulfillment of
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Department of Biological Sciences
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PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS OF *POLYMESODA EXPANSA* (CLAM) IN PCR AMPLIFICATION.Oleh Norasma binti Zakaria, No. Matrik UK 7858 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.

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%	Percentage
°C	Degree Celcius
µl	Microlitres
bp	Base pair
CPS III	Carbamoyl phosphate III
DNA	Deoxyribonucleic Acid
dNTP	2'-deoxynucleotide triphosphate
EDTA	Ethylenediaminetetracetic acid
g	Gram
kb	Kilobyte
mg	Miligram
$MgCl_2$	Magnesium chloride
ml	Mililitres
mM	Milimolar
NaCl	Natrium Chloride
ng	Nanogram
nm	Nanometer
OD	Optical Density
PCR	Polymerase Chain Reaction
pH	'Potential of hydrogen'
RAPD	Random Amplified Polymorphic DNA

RFLP	Restricted Fragment Length Polymorphism
RNA	Ribonucleic Acid
rpm	Round per minutes
SDS	Sodium Dedocyl Sulphate
TBE	Tris-Borate-EDTA buffer
TE	10 mM Tris-Cl, 1 mM EDTA
TNES- Urea	Tris – base, NaCl, EDTA, SDS – Urea
UV	Ultra violet
V	Volts
VDS	Video Documentation System

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ABSTRACT

The objectives of this study were to measure the purity and quantity of *Polymesoda expansa* DNA from different preserved tissue and to determine the best technique for DNA extraction of *Polymesoda expansa*. This study was also conducted to determine whether the genomic DNA that extracted can be used for PCR amplification. About 15 individuals of *Polymesoda expansa* was collected from Pulau Che Mansor, Setiu Wetland, Terengganu. The muscle tissue of *Polymesoda expansa* was storage in deep freezer, preserved in TNES-Urea buffer and 95 % Ethanol for one, two and three month. The genomic DNA of *Polymesoda expansa* were extracted using Wizard Genomic DNA Purification Kit (Promega) and Phenol: Chloroform extraction methods. The best preservative and DNA extraction was determine using the 1 % agarose gel electrophoresis and UV-Spectrophotometer. For PCR amplification, the RAPD-PCR technique was used. The primers that have used were OPA 01 to OPA 10. The best preservative was TNES-Urea buffer and the best DNA extraction was Wizard Genomic DNA Purification Kit (Promega). The entire of genomic DNA band from TNES-Urea buffer preserved tissue that extracted using the Wizard Genomic DNA Purification Kit (Promega) has the clear band. The quantity of genomic DNA from TNES-Urea buffer preserved tissue that extracted using Wizard Genomic DNA Purification Kit (Promega) was in the range of 340 to 612.5 (ng/µl) and the purity was in the range of 1.088 to 1.211 (OD 260/OD 280). The entire genomic DNA that obtains from different preserved tissue and extracted using the Wizard Genomic DNA Purification Kit (Promega) was suitable for using in PCR amplification.

PENGAWET DAN KAEDAH PENGEKSTRAKAN DNA YANG BERBEZA UNTUK TISU *Polymesoda expansa* (KEPAH) DALAM KAJIAN AMPLIFIKASI PCR

ABSTRAK

Objektif kajian ini ialah untuk mengukur ketulenan dan kuantiti DNA daripada tisu awet yang berbeza dan untuk menentukan kaedah pengekstrakan DNA yang paling baik bagi *Polymesoda expansa* untuk amplifikasi PCR. Sebanyak 15 individu *P. expansa* telah dipungut dari Pulau Che Mansor, Tanah Benchah Setiu, Setiu, Terengganu. Tisu otot *P. expansa* disimpan di dalam peti sejuk, diawet dengan menggunakan penimbal TNES-Urea dan 95 % Ethanol untuk satu, dua dan tiga bulan. DNA genomik *P. expansa* diekstrak menggunakan kaedah Wizard Genomic DNA Purification Kit (Promega) dan Phenol:Chloroform. Pengawet dan teknik pengekstrakan terbaik ditentukan menggunakan elektroforesis gel agarose dan UV-Spectrophotometer. Untuk amplifikasi PCR, kaedah RAPD-PCR digunakan. Primer yang digunakan ialah OPA 01 hingga OPA 10. Pengawet yang paling baik ialah penimbal TNES-Urea dan kaedah pengekstrakan genomik DNA yang paling baik ialah kit Promega. Kesemua genomik DNA yang diekstrak daripada tisu yang diawet di dalam penimbal TNES-Urea dengan menggunakan kaedah kit Promega mempunyai jalur yang jelas. Kuantiti genomik DNA daripada tisu yang diawet di dalam penimbal TNES-Urea dan diekstrak dengan menggunakan kit dalam julat 340 ke 612.5 (ng/ µl) dan ketulenan berada dalam nisbah 1.088 to 1.211 (OD 260/OD 280). Semua genomik DNA yang diekstrak daripada tisu awet yang berbeza dengan menggunakan Kit Promega sesuai untuk amplifikasi PCR.