THE USE OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *PINCTADA SP.* (PEARL OYSTER) IN PCR AMPLIFICATION STUDY

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Lihat sebelah

THE USE OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *PINCTADA SP.* (PEARL OYSTER) IN PCR AMPLIFICATION STUDY

By

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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 *PINCTADA SP.* (PEARL OYSTER) IN PCR AMPLIFICATION STUDY oleh Chong Lie Hien, no. matrik: UK 7748 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
DNA	Deoxyribonucleic Acid
°C	Degree Celsius
g	Gram
ml	Mililitre
ng	Nanogram
mg	Milligram
µl	Microlitre
rpm	Revolution per Minute
M	Molar
EDTA	Ethylenediamine Tetra-acetic Acid
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
1×	One time
kb	Kilobase
G	Guanine
C	Cytosine
OD	Optical Density
bp	Base pair
nm	Nanometer

UV	Ultra Violet
TNES	Tris-NaCl-EDTA-SDS

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ABSTRACT

This study was aim to measure the purity and quantity of DNA *Pinctada sp.* from TNES-urea buffer and 95% ethanol and to compare the efficiency of Wizard Genomic DNA Purification Kit (Promega) and phenol-chloroform extraction for DNA extraction. Through PCR amplification, there are positive amplifications from only TNES-urea buffer preserved tissue; almost no amplifications were made from 95% ethanol-preserved tissue. The experiment was designed in laboratory conditions over a period of one to three-month, testing the preservatives TNES-urea buffer and 95% ethanol. Five replicates of each preservation treatment were stored at room temperature, while control samples were cryopreserved. DNA was extracted using Promega kit and phenol-chloroform extraction, and quality was assessed by electrophoresis on agarose-gel followed by PCR amplification. Results show that the length of time in storage has no much affected the presentation of DNA. TNES-urea buffer are significantly better than 95% ethanol for high quality DNA preservation using phenol-chloroform extraction. With the ratio of 1.8 as a standard for pure DNA, the phenol-chloroform extraction produce a better purity and quantity of DNA than Promega kit, in where the purity of genomic DNA were range from 1.187 to 2.015 with the quantity of DNA from 593.4 to 1007.5 ng/μl.

PENGGUNAAN DUA JENIS PENGAWET DAN KAEDAH PENGEKTRAKAN DNA YANG BERBEZA DARIPADA TISU-TISU *PINCTADA SP.* (TIRAM MUTIARA) DALAM KAJIAN AMPLIFIKASI PCR

ABSTRAK

Kajian ini bertujuan untuk menilai puriti dan kuantiti DNA *Pinctada sp.* daripada TNES-urea buffer dan etanol 95%, dan membandingkan keberkesanan Wizard Genomic DNA Purification Kit (Promega) dan pengektrakan phenol-chloroform dalam pengektrakan DNA. Melalui amplifikasi PCR, hanya tisu yang diawet dalam TNES-urea buffer menunjukkan amplifikasi yang positif, hampir tiada amplifikasi daripada tisu yang diawet dalam etanol 95%. Eksperimen ini dilakukan dalam keadaan makmal bagi tempoh satu hingga tiga bulan, untuk menguji pengawet TNES-urea buffer dan etanol 95%. Lima sampel dari setiap pengawetan telah disimpan dalam suhu bilik, manakala sampel kawalan disejukbekukan. DNA telah diekstrak dengan menggunakan kit Promega dan pengekstrakan fenol-klorofom, dan kualiti ditentukan oleh elektroforesis atas gel agaros diikuti oleh amplifikasi PCR. Keputusan menunjukkan Tempoh masa penyimpanan tidak banyak mempengaruhi kehadiran DNA. TNES-urea buffer jelas lebih baik daripada etanol 95% untuk pengawetan DNA yang berkualiti tinggi dengan menggunakan pengekstrakan fenol-klorofom. Dengan nisbah 1.8 sebagai piawai untuk DNA tulen, pengekstrakan fenol-klorofom menghasilkan puriti dan kuantiti DNA yang lebih baik daripada kit Promega, di mana puriti genomik DNA adalah dalam lingkungan 1.187 hingga 2.015 dengan kuantiti DNA dari 593.4 hingga 1007.5 ng/μl.