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Comparison of Simple and Rapid DNA extraction procedures for *Tridacna squamosa*

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Abstract: There are a lot of methods for isolation of genomic DNA but some are time-consuming, expensive and requires higher amount of tissue. *Tridacna squamosa* belongs to the CITES listed species therefore a finding the most practical and reliable DNA extraction method is important. This study compared Kapa Express Extract protocol with previously published CTAB and chelex method for DNA extraction of *T. squamosa* and used a field-friendly mini PCR machine for DNA amplification. The optimized KAPA express protocol is better due to: (1) higher DNA yield; (2) lesser time of extraction (3) environmental friendly where less waste products are involved. This study presents a simple, rapid, economical and field friendly method for DNA isolation of *T. squamosa.*

Key Words: One-Tube Extraction; *Tridacna*; Giant Clams; Philippines; DNA Extraction

1. INTRODUCTION

The family Tridacnidae or commonly called Giant Clams are protected under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) from 1985. In the Philippines, Giant clams or locally known as Taklobo are regulated by the Philippine Fisheries code, FAO 208 and RA 8550. The Philippine law prohibits collection of living specimens, raw shells, meat consumption and any byproducts of Giant Clams.

The Bureau of Fisheries and Aquatic Resources (BFAR) is the main regulating body of marine and aquatic species in the Philippines. The bureau only allows the collection of giant clams from the wild for research purposes where the proponent must comply with the requirements under the Fisheries Administrative Order 233. The requirements will be under discussion and approval of the BFAR-Philippine Aquatic Red List Committee (PARLC). Pursuing the permit is a long and tedious process where the proponent needs to get the permission of the Local Government Units. All of the local Agriculture Officers are fully aware that the giant clams are CITES listed species and mostly require not to kill the clams when research will be conducted.

Given the requirements for permits and the value of giant clam tissue, a prudent laboratory procedure for DNA extraction for further downstream procedure has to be developed. The researcher will only collect the smallest possible amount of tissue. This is to ensure that the clams will still be alive throughout the study. It is also a way to propose to the Agricultural Officer that the research will not harm any Tridacnidae species, therefore, a better way to get the permits. This study compared the efficiency of DNA extraction yield and PCR success rate of 3 different DNA extraction

methods for *Tridacna Squamosa* from the Dumaguete, Philippines.

2. METHODOLOGY

2.1 Sample Collection

Tridacna squamosa tissues were collected from the cultures of Silliman University- Marine Laboratory in Dumaguete, Philippines. Morphological identification was done with FAO species identification guide volume 1 (Carpenter and Niem 1998). A small amount of tissues were cut, as small as one No. 35 staple wire, and was preserved with Phosphate Buffered Saline (PBS) in a 1.5-ml microcentrifuge tube. Voucher specimen was initially stored in an ice chest and was transferred to -20°C freezer in DLSU-PGL laboratory.

Figure 1. Philippine Map showing Dumaguete

2.2 DNA Extraction

The procedures were compared and summarized processes are listed below:

2.2.1 KAPA Express Extract

A ~2 mm^3 fragment of *T. squamosa* tissue section was placed in a new 1.5 ml microcentrifuge tube with KAPA Express Extract according to the manufacturer's instruction. The incubation period was modified in this study compared with the manufacturer's protocol. The microcentrifuge tube with tissue was incubated for 15 minutes at 75°C using dry bath. Second incubation was done for 10 minutes at 95°C. After incubation, the tube was centrifuge at high speed for 1 minute. The supernatant was transferred to a fresh tube.

Figure 2. *Tridacna squamosa* showing mantle (top view)

2.2.2 Chelex® 100 Molecular Biology Grade Resin |

The Chelex protocol was done using modified method from Lagman and Cruz-Abeledo (2017). A 300 µL of freshly prepared 10% Chelex solution was pipetted in a sterilized microcentifuge tube. A giant clam tissue with approximately 1/3 of the length of a staple wire was placed in the extraction buffer. The tube was incubated at 90 °C for 30 min in a dry bath and was directly submerged in ice after heating. The tube was centrifuged at 12,000 rpm for 5 min and the supernatant was placed in a sterilized tube.

Figure 3. *Tridacna squamosa* showing scutes

2.2.3 CTAB Extraction Buffer

CTAB extraction procedure was performed based from Neo (2013) with modifications. Giant clam tissue samples, 600 µL freshly prepared CTAB buffer and 20 µL ml-1 proteinase K was incubated for 24 hours at 55°C. After incubation, 500 µl phenol/chloroform mixture was added to the tube and vigorously shaken for 30 seconds. The tube was centrifuged at 6,000 rpm for 1 min before obtaining the aqueous supernatant. The whole process was repeated to obtain an equal volume of absolute ethanol. The samples were stored at -80 °C overnight to reveal the DNA pellet. A 70 % ethanol was used to wash the pellet.

2.3 PCR Amplification

mtDNA CO1 gene. The universal primer from Folmer et al. (1994) was used in this study. Polymerase Chain Reaction (PCR) reactions were performed in a total volume of 25 µL containing approximately: 1 µL DNA template, 17.1 µL ddH_2O, 5 µL 5X KAPA Taq Buffer, 1.5 µL 25MM $MgCl_2$, 0.5 µL 10 MM dNTPs, 0.4 µL of each primers (10µM). The miniPCR™mini8 thermal cycler were used.

3. RESULTS AND DISCUSSION

The Kapa Express Extract has the least amount of time for DNA extraction compared with the other protocols. CTAB buffer is inconvenient for on-site or field surveys because the procedure requires 1-2 days for DNA extraction. In regards to DNA yield, Kapa Express Extract has a better yield than the rest and as a result, bands are also present with Kapa.

Table 1. Comparison of the Different DNA Extraction Method

	Kapa Express Extract	Chelex Buffer	CTAB Buffer
Time	~30 min	~40 min	~1-2days
DNA yield (ug/uL)	1.8	.06	1.2
PCR success	DNA bands present	DNA bands absent	Dimers present
Use of Proteinase K	None	Yes	Yes
Use of phenol/chloroform mixture	None	None	Yes
No. of Tubes/Sample	1-3	1-3	1-3

Apparently, Kapa Express Extract is a better method for one-tube DNA extraction for *Tridacna squamosa.* This procedure is ideal if a researcher has huge number of samples or needs multiple batch of extraction. Also, the Kapa protocol can be suitable if one would intend to directly perform DNA extraction on-site during fieldworks. Mini PCR is still not widely used technology, but the current study used the mini PCR and it works with the optimized Kapa protocol.

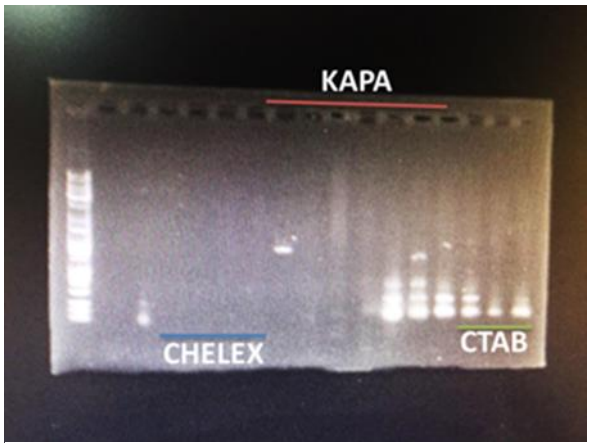

Figure 4. Gel Electrophoresis Results

4. CONCLUSIONS

The optimized KAPA express extract one-tube protocol in this study has a better DNA yield and PCR success than the CTAB and Chelex extraction procedure. The success of one-tube protocol combined with the use of mini-PCR can be more efficient and useful for DNA extraction and PCR directly in the field.

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6. REFERENCES

Carpenter, K. E. and V. H. Niem. (1998). FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 1. Seaweeds, corals, bivalves, and gastropods. Rome, FAO.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol, 3: 294-299.

Lagman, M. C. and Cruz-Abeledo, C.C.V. (2017). DNA Barcoding for Marine Organisms. De La Salle Universit Publishing House, 2017 p. 13-15.

Neo M. L. (2013) Giant clams (Subfamily Tridacninae) in Singapore: Past, Present, and Future. ScholarBank@NUS Repository. doi: http://scholarbank.nus.edu.sg/handle/10635/48638