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Comparative Evaluation of Different Established Genomic Extraction Methods for Scleractinian Coral

M.I.A. Ghafari, R. Agus* and M. Litaay

Department of Biology, Faculty of Mathematic and Natural Sciences, Hasanuddin University. Jl. Perintis Kemerdekaan No.KM.10 Makassar 90245, South Sulawesi Province, Indonesia

Studies on coral genetics have been widely used recently for varied research purposes, including to solve the taxonomical challenge or to understand the physiological-ecological aspects of corals towards climate change. While the information provided by coral genome is crucial for those works, isolation of coral genome is still a difficult preliminary step due to coral's nature. This study aimed to evaluate and to suggest the best application of among four established methods for DNA extraction from scleractinian coral, i.e., Guanidine hydrochloride (GuHCl), Chelex chelating resin, Guanidinium thiocyanate-phenol-chloroform (AGPC), and spin-column methods. Coral samples were grounded and submitted to each extraction method's protocol. Final extracts were checked for their quality and quantity. The final extracts feasibility were tested through PCR (Polymerase Chain Reaction), followed by electrophoresis for amplicon quality screening. The scoring was done for assessment aspects: time efficiency, successful extraction process, extracted DNA quantity and quality, final extract properness, and cost effort per sample. The highest DNA concentration was obtained by spin-column methods, reaching up to 724.31 ng/µl, while other methods can only provide between 264.94±36.453 to 378.19±0 ng/µl of DNA. Among all method tested, the spin-column was able to provide reproducible and feasible quality of final extracts for the amplification. Other methods were failed to provide such consistent and proper results. The results suggest that the spin-column is the most appropriate method for DNA isolation from scleractinian specimen.

Keywords: Coral genetic; DNA extraction; electrophoresis; polymerase chain reaction; spin-column method

I. INTRODUCTION

Corals are key species that form the basis of coral reef ecosystems. Those ecosystems are home for about 25% of marine species (Hoegh-Guldberg *et al.*, 2019), and also provide a huge ecosystem services such as coastal protection, oxygen, food and income sources for about 1 billion people on earth (Rivera *et al.*, 2020). Conservation of coral reef ecosystem is so important, that which majority of current biological marine study focuses on that topic. Countries with the highest coral reef biodiversity, such as Indonesia, Philippines and the Caribbean countries, are struggling for coral reef conservation to prevent the threat of coral extinction due to climate change.

Genetic approaches have been carried out so far for taxonomic identification purposes or to understand the physiological-ecological aspects of corals towards the threat of climate change. Coral's genome have provided a better tool for discovering coral cryptic species (Quattrini *et. al.*, 2019; Gómez-Corrales & Prada 2020; Oury *et al.*, 2020), as well as a solution for confused taxonomical and phylogenetic among coral family group 'the Bigmessidae' (Huang *et al.*, 2011). In addition, genetic diversity and genotype factors are crucial in determining the adaptive capacity, risk of extinction and resilience level among corals (Barshis *et. al.*, 2013; Bay *et al.*,

*Corresponding author's e-mail: rosanaagus@unhas.ac.id

2017). The high frequency of bleaching in the last decades has significantly reduced the level of coral genetic diversity, which decline the coral capacity to survive and may lead to the future collapse of the coral reef ecosystem worldwide (Sully *et al.,* 2019). As an effort to prevent corals from extinction, recent conservation strategies are not only focused on protecting genetic diversity, but also have led to the use of genetic data for various advanced purposes, such as translocation and hybridisation of corals (van Oppen *et al.*, 2017), identification of adaptive coral-symbiont variants (Bachtiar *et. al.*, 2019; Chen *et al.*, 2020), and genome editing for super corals (Camp *et al.*, 2018).

While the information provided by coral genomics is critical to understand the aspects of corals adaptation, basic step such as purification of coral genome is still challenging issue and sometimes can be very tricky. In scleractinian corals, their tissues are covered by lime and other residual compounds that potentially act as inhibitors during the PCR (Barton *et. al.*, 2006; Schrader *et al.*, 2012), leaving the blank or biased results. The process of removing inhibitors and residual compounds during extraction could reduce the quality and quantity of eluted DNA (Japelaghi *et al.*, 2011). Those processes also reported to decrease the efficiency and sensitivity of PCR, which leads to false-negative results (Schrader *et al.*, 2012). The presence of mesoglea connective tissue along with lime and other minerals will complicate the cell lysis process in the early stages of genome extraction (Weber *et. al.*, 2017; Bouchard *et al.*, 2020). Therefore, this study is a preliminary effort to evaluate the application of several established methods for DNA extraction from scleractinian coral.

II. MATERIALS AND METHODS

A. Tested Samples

The tested sample was a preserved specimen of coral *Echinopora lamellosa* in 95% EtOH from Ghafari (2021), which originated from Alas Strait, eastern Lombok waters, Indonesia. The sample was ground into a fine white powder stock for further extraction process. Furthermore, this sample stock will be called EL, which stands for *E. lamellosa*. A total of 3 samples (labelled as EL1, EL2, and EL3) were taken from the EL sample stock and to be submitted for each further extraction process.

There are four different extraction methods were tested in this study. Each method was previously used to extract genome from coral or marine invertebrate. The protocol of each method as explained in procedures section below.

B. Procedures

1. Guanidine Chloride (GuHCl) Extraction Method

This method has been used for extracting genomes of sponges (Salgado *et al.*, 2007), benthic foraminiferan (Sabbatini *et al.*, 2014) and acroporid corals (Fukami *et. al.*, 2000; Morgan *et al.*, 2001). The extraction procedure includes taking 0.15 mg of *Echinopora lamellosa* powder stock, then soaking it in a 1.5 ml tube containing 100% EtOH. The sample was centrifuged for 10 min, followed by adding 150 µl of GuHCl solution. The tube was incubated for 30 min at 55°C (vortex 3-4 times during heating). After the incubation, the sample was cooled at room temperature for 30-90 min. Then, the cooled tube was re-centrifuged for 2 min at 15,000 rpm. After that, the supernatant will be visible. As much as 100 µl of supernatant was transferred to into a new 1.5 ml tube, followed by adding 100 µl of isopropanol into the supernatant and vortexed until homogeneous. The mixture was stored overnight at -20°C. After that, the mixture was re-centrifuged for 15 min at 20,000 rpm and the appeared supernatant was discarded. Following the step, 100 µl 70% EtOH was added to the mixture and then vortexed to ensure the mixture was blended homogeneously. Subsequently, the mixture was centrifuged again for 5 min at 15,000 rpm, followed by the discarding of appeared supernatant. Finally, 30 µl ddH2O was added to the mixture. To ensure DNA was diluted and unclumped, the final extract was kept for 5 min at -5°C and 5 min at 55°C alternately for 3 times, before it being permanently stored at -20°C.

2. Chelex Chelating Extraction Method

Previously, this method was used to extract DNA from larvae of *Acropora palmata* (Baums *et al.*, 2005) and from adult coral specimens of *A. Hyacinthus* (Wijayanti *et al.*, 2018a, b), as well as from other marine benthic such as sea squirt *Polycarpa aurata* (Timm *et al.*, 2017) and giant clam *Trochus*

niloticus (Holman *et al.*, 2019). The extraction procedure includes taking 0.15 mg of *Echinopora lamellosa* powder stock, then soaking it in a 1.5 ml tube containing 10% Chelex 100 Chelation Resin for 90 min. Then, the soaked sample was vortexed for 10-15 s, continue with post incubation for 20 min at 95°C. The sample was re-vortexed to ensure that all impurities were suspended at the bottom of the tube. The supernatant was transferred carefully into a new 1.5 ml tube. The final extract was stored at -20°C.

3. Guanidinium Thiocyanate-Phenol-Chloroform (AGPC) Extraction Method

This method has been used for the genomic extraction of corals *Diploria strigosa* (Anderson & Gilchrist, 2008), *Stylophora pistillata* (Maor-Landaw *et al.*, 2017), *Pseudopterogorgia elisabethae* (Santiago-Vázquez *et al.*, 2006) and coral symbionts (Santiago-Vázquez *et. al.*, 2006; Rosic & Hoegh-Guldberg 2010). The AGPC method applied in this study utilises TRIzol™ by ThermoFisher Scientific. The working procedure includes taking 0.15 mg of *Echinopora lamellosa* powder stock and soaking it in a 1.5 ml tube containing 1 ml of TRIzol™, then vortex it until homogeneous. The mixture was incubated for 5 min in order to dissociate the cell nucleoprotein complexe. An extended incubation was done for 2-3 min, by adding 200 µl of chloroform. The mixture was then centrifuged for 15 min at a speed of 12,000 × g at 4°C. The mixture was turned into a red precipitate of phenol-chloroform at the bottom, the interphase and the clear aqueous portion are at the top. The aqueous portion was discharged and the interphase portion (which contains DNA) was transferred into a new 1.5 ml tube. Then, 300 µl of 100% EtOH was added and shaken gently into the interphase mixture, followed by short incubation for 2-3 min. Centrifugation for 5 minutes at a speed of 2,000 × g at 4°C was done to separate and discard the supernatant, which contains the protein residue. Finally, the final extract was diluted with 100 µl of ddH2O and stored at -20°C.

4. Spin-Column Kit Extraction Method

The gSYNC™ DNA Extraction Kit by Geneaid and the DNeasy® Blood & Tissue Kit by Qiagen were used separately to extract coral DNA according to the manufacturer's instruction with overnight incubation. Similar to other methods, 0.15 mg of *Echinopora lamellosa* powder stock was used for DNA extraction using this method.

C. Data Analysis

The final extracts through each method were checked quantitatively and qualitatively. The quantity check of final extracted DNA was carried out using SmartSpec Plus (Bio-Rad). The quality of the extracted DNA was visualised by using electrophoresis method. Electrophoresis for final extract was carried out with 1% agarose gel at 100 V for 45 min.

Furthermore, PCR was carried out to determine the suitability of final extract for amplification using specific primers ITS1F (5'-CTTGTTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Gardes & Bruns 1993) to amplify the whole ITS (Internal Transcribed Spacer) fragment. As much as 0.5 µl of each primer were added to the master mix containing 12.5 µl of Taq DNA Polymerase (MyTaq™ HS Red Mix, Bioline®), 9 µl of ddH2O (SuperPure nuclease-free water, BioScience®) and 2 µl of DNA template. The amplification was performed with 35 cycles: 45s at 94°C, 30s at 47°C, and 1 min at 72°C. The quality of amplicon was observed visually by electrophoresis on 1.5% agarose gel with 1 kb DNA ladder. The electrophoresis was run at 100 V for 45 min. Good quality amplicons are identified by thick and clear DNA bands.

Data assessment was carried out by assigning a score in the range of 1 to 3 for several evaluation aspects, which cover time efficiency, quality and quantity of DNA isolates, final extract feasibility for amplification and estimated overall cost per sample as shown in Table 1. Final conclusions were then made descriptively on the extraction protocol with the highest total score.

III. RESULTS AND DISCUSSION

The result of this study demonstrates the difference efficacy by four different established DNA extraction protocols that have been used previously for genetic studies of various corals and other marine benthic. Different extraction methods may produce vary results in terms of efficiency and reproducibility, depending on the challenge encountered during the extraction process, which can be originated from

any physical or chemical matrix of the sample. Of those four different DNA isolation protocols tested, calculations on various evaluation aspect other than their quality and quantity of extracted DNA also showed different performances, which can be utilised as a basis for consideration in selecting proper DNA isolation techniques to be applied.

Table 1. Parameter score for extraction quality assessment

Score	Evaluation aspect					
	Time efficiency	**Successful extraction**	**Extracted DNA quantity**	**Extracted DNA quality**	**Final extract properness for PCR**	**Cost**
1	>24 h	1/3 samples	<200 ng·µl^{-1}	A260/A280<1.80	1/3 samples	Expensive (> 5 USD (~>71,206 IDR)/sample)
2	12-24 h	2/3 samples	200-400 ng·µl^{-1}	1,80≤A260/A280≤2.00	2/3 samples	Moderate (between 2.5 to 5 USD (~35,603 to 71,206 IDR)/sample)
3	<12 h	3/3 samples	>400 ng·µl^{-1}	A260/A280>2.00	3/3 samples	Inexpensive (< 2.5 USD (~<35,603 IDR)/sample)

Investigation on the workability aspect of different established method for coral DNA extraction proves that, among those four methods tested, both spin-column methods (gSYNC and DNeasy) performed better and consistent results. Spin-column method seems to be optimal for coral's genome extraction by successfully extracted DNA from 3/3 sample, as presented in Figure 1. All final extracts through spin-column method were successfully amplified. The DNA quality and concentration obtained in the final extracts through spin-column methods were considerably higher than produced by other methods, which is evidenced by the thick and clear DNA band shown in Figure 1. As presented in Table 2, the DNA purity value obtained by all sample extracted using spin-column method are above 1.8, which means that no suspected non-genomic material contamination present, such as phenol or protein (Lucena-Aguilar *et al.*, 2016).

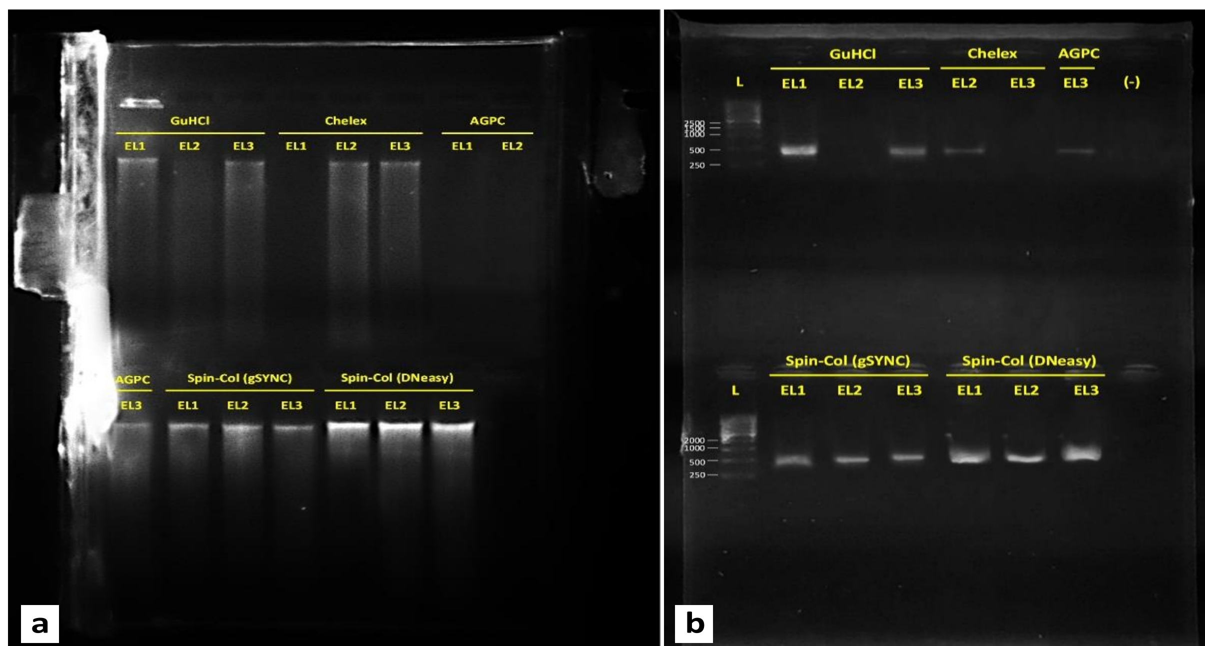

Figure 1. Qualitative checking of (a) all final extract by electrophoresis, and (b) amplicon by electrophoresis

Table 2. Parameter score for extraction quality assessment

Method	Sample Code	Wavelength (A260/A280)	DNA Concentration (ng/µl)	Average DNA Concentration (ng/µl)
GuHCl	EL1	2.18	293.05	264.94±36.453
	EL2	1.54	203.92	
	EL3	2.09	243.85	
Chelex	EL1	N/A	N/A	271.99±32.790
	EL2	1.69	304.78	
	EL3	1.86	239.20	
AGPC	EL1	N/A	N/A	378.19±0
	EL2	N/A	N/A	
	EL3	1.76	378.19	
Spin-Col:				
a. gSYNC	EL1	2.33	397.66	366.22±50.424
	EL2	2.15	295.07	
	EL3	2.03	405.93	
b. DNeasy	EL1	2.42	491.23	538.42±136.650
	EL2	2.23	399.72	
	EL3	2.19	724.31	

The results of this study indicate that different established extraction methods have different efficacy, efficiency and reliability for extracting coral's DNA. The final extract obtained through spin-column method give a good reproducible amplicon, with the evaluation score of 15 points, while other method has a lagging score below it, as shown in Table 3. The spin-column method utilises the principle of separating molecules based on their affinity by binding the genomic material to a solid-phase (usually silica-based matrix) under chaotropic conditions, followed by the removal of non-genomic material with a certain appropriate buffer solution and pulling the genomic material from solid-phase with a low-salt solution such as purified water (diH_2O, dH_2O or ddH_2O) or Tris-EDTA (Shi *et. al.*, 2018; Dairawan & Shetty, 2020). Through the affinity based-molecular binding mechanism, it is thought to provide advantages to obtain DNA with a higher concentration and purity level than other methods. Although there are differences in the solid phase matrix material, where gSYNC uses a glass fibre-based membrane (Geneaid, 2021), while DNeasy uses a silica-based membrane (Qiagen, 2021), this study does not confirm any differences in the feasibility of the extraction results from the two kits for the downstream process. On the other hand, even though the spin-column method seems to surpass other methods, those method is more expensive and time-consuming.

Table 3. Evaluation score for DNA extraction method

No	Extraction method	DNA extraction effectiveness score*						Total score
		Time efficiency	Successful extraction	Extracted DNA quantity	Extracted DNA quality	Final extract properness for PCR	Cost	
1	GuHCl	2	3	2	2	2	3	14/15
2	Chelex	3	2	2	1	1	3	12/15
3	AGPC	3	1	2	1	1	3	11/15
4	Spin-col:							
	a. gSYNC	2	3	2	3	3	2	15/15
	b. DNeasy	2	3	3	3	3	1	15/15

The reproducible results did not occur in other final extract coming from other extraction methods. Both GuHCl and Chelex methods gave final extracts that looked promising at the beginning. Unfortunately, not all of those extracts were able to be amplified. The results produced through GuHCl methods almost comparable with the final extract using Chelex, whereas the basic substance and the extraction mechanisms of both methods are very different. GuHCl is a dangerous compound that acts as a lysis buffer, as well as being a chaotropic salt for denaturing the impurity proteins and RNA (Yaffe *et al.*, 2012). In the other hand, Chelex does not contain harmful compounds (Ali *et al.*, 2017). Chelex keeps the released DNA undamaged from lysed cell, by binding the magnesium ions which is a DNAse co-factors (Singh *et al.*, 2018).

Apparently, neither GuHCl nor Chelex were effective enough to produce a feasible final extract with reproducible quality for PCR from complicated specimens, such as coral. Although both methods have been used for isolation of coral DNA by Fukami *et al.* (2000), Morgan *et al.* (2001), Baums *et al.* (2005), and Wijayanti *et al.* (2018a; b), none of these studies intended to confirm the success rate of their extraction process. In terms of extraction efficiency, GuHCl takes the longest time among the four method tested, while Chelex only takes about 2 hours to complete the extraction work. Despite many drawbacks displayed by both methods, those are considered very cheap for extraction cost per sample unit. This aspect can be taken into consideration and the low cost allow researcher to repeat the extraction process to get the feasible final extract for amplification.

This study also shows different quality of final extract obtained through GuHCl and AGPC method, though both method use the role of Guanidine compounds (CH_5N_3) as chaotropic agents. In this study, TRIzol reagent was used in AGPC method, a hazardous acid solution which consists of Guanidinium thiocyanate (GuSCN) acid, sodium acetate and a mixture of phenol-chloroform. The basic principle of using TRIzol reagent is to separate RNA from DNA and protein, by binding the DNA and protein molecules in an interphase or lower phase of the extract solution (Ali *et al.*, 2017). The AGPC method has provided a poor final extract in this study. We suspect that the poor result was due to contamination of non-genomic substance during the pipetting step, as the only sample that was successfully extracted still managed to provide even a faded band. Shu *et al.* (2014) stated that the extraction protocol using AGPC method can be uncertain and differs for each specimen, where incompatibility between specimen-procedures may resulted in none of samples was successfully extracted. Therefore, the use of AGPC method in extracting coral's genome could not be taken into consideration yet, where the possibility of accidentally taking contaminant precipitate during pipetting needs to be tested for further clarity.

It is suspected that the process of separating genomic and non-genomic molecules plays an important role in increasing the concentration and purity of the extracted DNA, as well as contribute to the addition of contaminants. The GuHCl, Chelex and AGPC methods utilise manual separation by pipetting to obtain genomic molecules. Separation through pipetting runs the risk of wasting the desired genomic molecule. Pipetting also give a possibility for non-genomic material accidentally be taken, which can reduce the DNA purity of the final extract and inhibit the PCR process (Bánkuti *et al.*, 2020), leading to inconsistent amplification results or even thwart the amplification process (Lienhard & Schäffer, 2019).

Selection of the most appropriate method for extracting coral genomes is a very important step prior to any other further process and analysis. Despite the fact that this study shows the superiority of the spin-column method for coral DNA extraction, researchers can choose other methods in terms of cost, equipment availability and time efficiency. Based on the comparative evaluation tested in this study, we recommend the spin-column method as the most indicative method for extracting coral genomes.

IV. CONCLUSION

In conclusion, this study shows commercial spin-column kit (gSYNC and DNeasy) has been outperformed other extraction methods in providing reproducible and feasible extracted genomic material from coral specimen. The use of GuHCL or Chelex for isolating corals DNA are notably inexpensive, but the quality of the final results are inconsistent and could lead to doubtful downstream process. In the other hand, one may choose those methods for cost and time efficiency reason. The DNA extraction method has

become a very decisive factor in determining the quality of final output and by considering all compared aspect to evaluate four methods tested in this study, we recommend the spin-column method as the most indicative method for extracting coral genomes.

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