DNA extraction and pattern of crab and macrobentos from North Sumatran mangrove forest, Indonesia

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Abstract. Mangrove forest ecosystem is one of the most productive and unique ecosystems that serves to protect coastal areas from various disturbances, as well as provide habitat for various animal species. The large number of crab species and macrobentos in mangrove ecosystems results in frequent errors in the naming of species that have similarities in morphological features. This problem can be solved through a comprehensive approach by combining morphological analysis with genetic analysis. This study aims to report a DNA extraction and PCR amplification prior was used for the identification of crab and macrozoobentos from mangrove forest, North Sumatra. Primer 16S has a conserved area so it is appropriately used in Polymerase Chain Reaction (PCR) and sequencing analysis to determine taxonomy, phylogeny and diversity between species. Visualization of PCR amplification results with primer16S from crab samples and macrobentos resulting a low DNA band with a size of 206 bp and a high of 678bp

1. Introduction
Mangrove forest ecosystem is one of the most productive and unique ecosystems that serves to protect coastal areas from various disturbances, as well as provide habitat for various animal species [1]. Especially detritivore animals that live and associate in mangrove ecosystems [2]. The large number of crab species and macrobentos in mangrove ecosystems results in frequent errors in the naming of species that have similarities in morphological features. This resulted in the establishment of a different species of the same name.

The phenomenon of cryptic species in marine biota is a problem that needs to be solved through a comprehensive approach by combining morphological analysis with genetic analysis [3]. Identification of organisms was originally based solely on morphological characteristics, but this time has led to molecular taxonomy in which an organism is grouped based on its gene similarity [4]. This study aims to report a DNA extraction and PCR amplification prior was used for the identification of crab and macrobentos from mangrove forest, North Sumatra.
2. Materials and methods

2.1. Crab and macrozoobentos material
The genetic material used is crab DNA and macrobentos from mangrove forest obtained from Percut, Belawan, Lubuk Kertang, Pulau Sembilan and Langsa, North Sumatera Province. The specific primer used is 16S primer. 16S has a conserved area so it is appropriately used in polymerase chain reaction (PCR) and sequencing analysis to determine taxonomy, phylogeny and diversity between species [4].

2.2. DNA Extraction
DNA isolation using Reliaprep gDNA Tissue Miniprep System kit protocol. Briefly, in the extraction process the parts used are meat from crab samples and macrobentos. The part is then added liquid nitrogen and then ground using mortar. The sample was transferred to a 1.5 ml tube then adds 100 µl Tail Lysis Buffer and 20 µl Proteinase K Solution, then vortex for 10 sec. After that, added 200 µl Cell Lysis Buffer and vortex for 10 sec. The sample was incubated at 56°C for 30 minutes, the sample alternates every 10 minutes. The 20 µl RNase A and vortex and sentrifuged 10,000 rpm for 10 minutes. After that separated part of the liquid with the garbage that settles. Then added 250 µl Binding Buffer, then sentrifuged 10,000 rpm for 5 minutes. is then transferred the top of the liquid to the filter tube, then centrifuge 10,000 rpm for 2 minutes. After that added 500 µl Column Wash Solution and then sentrifuge 10,000 rpm for 2 minutes. This activity was carried out as many as 3 repetitions. Column Wash Solution fluid in the tube. Then put tube 1.5 ml under the filter tube, then added 100 µl Nuclease Free Water sentrifuge 12,000 rpm for 1 minute. The extraction results are stored in the freezer for 1 night [5].

2.3. Polymerase Chain Reaction (PCR)
PCR mix was made with a combined solution component of dna dilute 2 µl, Primer forward and reserve respectively 1 µl, ddH2O 3.5 µl and Green Go Taq 2.5 µl. Amplification is done with PCR machine. PCR program is divided into: pre denaturation for 2 minutes at 94°C, denaturation for 30 seconds at 94°C, annealing for 1 minute at 56°C, extension for 3 minutes at 72°C, final extension for 7 minutes at 72°C and storage for 30 minutes at 4°C as previously described [6].

2.4. Agarose Gel Electrophoresis
PCR products were analyzed with electrophoresis in performed by voltage 70 V, current 220 A for 45 minutes in a 1% agarose gel stained with a Red Gel. Electrophoresis results are visualized using uv transmilators.

2.5. Data analysis
Amplification of DNA readings was analyzed by software UV-1D (UV-Tex verv16,09b) as earlier suggested [7]

3. Results and Discussion
Table 1 shows the length of DNA fragments viewed with UV-ID after irradiation with UV light. The length of DNA fragments for crab and macrobentos from forest mangroves ranged from 206-678.
Table 1. DNA amplification of Crab and Makrobentos from Mangrove Forest of North Sumatera.

| Line | Access Code | Amplicon (bp) |
|------|-------------|---------------|
| 1    | LGS 1 (Crab)| 489           |
| 2    | LGS 2 (Crab)| 277           |
| 3    | BLW 6 (Makrobentos) | 292         |
| 4    | LGS 3 (Makrobentos) | 247         |
| 5    | PCT 5 (Makrobentos) | 300         |
| 6    | LGS 5 (Crab) | 289           |
| 7    | LK 5 (Makrobentos) | 230         |
| 8    | PS 5 (Makrobentos) | 269         |
| 9    | PS 2 (Makrobentos) | 341         |
| 10   | PS3 (Crab) | 284           |
| 11   | LK 4 (Makrobentos) | 234         |
| 12   | LGS 9 (Makrobentos) | 219         |
| 13   | PCT 7 (Makrobentos) | 206         |
| 14   | PS 6 (Makrobentos) | 678         |
| 15   | BLW 9 (Makrobentos) | 423         |
| 16   | LGS 8 (Crab) | 360           |
| 17   | PCT 3 (Crab) | 227           |
| 18   | BLW 10 (Crab) | 227           |
| 19   | BLW 8 (Makrobentos) | 378         |
| 20   | PS 7 (Makrobentos) | 489         |
| 21   | PC 4 (Makrobentos) | 388         |

Description: LGS (Langsa), PCT (Percut), BLW (Belawan), PS (Pulau Sembilan), LK (Lubuk Kertang).

![PCR Results of Crab DNA and Macrobentos from Mangrove Forest, North Sumatera](image)

**Figure 1.** PCR Results of Crab DNA and Macrobentos from Mangrove Forest, North Sumatera

L = Ladder, lines 1-10 = PCR results (b) L = Ladder, lines 11-21 = PCR results documented with UV-1D software (UV-Texver v16.09b).

Visualization of PCR amplification results with primer16S of crab samples and macrobentos produces thin ribbons and bright and clear ribbons with different values from each sample (Figure 1). The amplification stage produces a low DNA band with a size of 206 bp and a height of 678 bp. DNA amplification from crabs and macrobentos obtains a single and multi band where a single band indicates...
that primary used is the primary that specifics to amplify crab DNA and Macrobentos [8]. While, multiband or double tape is suspected because that primer used is not specific to crabs and makrobentos [9]. DNA bands produced through electrophoresis can be analyzed to see the genetic diversity of a group organism [10].

4. Conclusion
DNA crab and macrobentos were successfully amplified using primary 16S which produced a number of different DNA patterns in each sample ranging from 206 -678 bp and further sequenced for further analysis.

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