# NOVEL APPROACHES TO MOLECULAR DIAGNOSTICS OF *PROROCENTRUM MINIMUM* IN THE GOLDEN HORN ESTUARY, THE SEA OF MARMARA

I. Uzonur [1]*, F. Koyuncu [1], S. Tas [2], Z. Ulupinar [1] and E. Muftuoglu [2]
[1] Fatih University, Biology Department - iuzonur@fatih.edu.tr
[2] Istanbul University, Institute of Marine Sciences and Management, Physical Oceanography and Marine Biology Department, Istanbul, Turkey

## Abstract
The identification and quantification of individual algal species remains difficult, time consuming, requiring a great deal of expertise and experience. Alternative to traditional, morphology-based methods, molecular techniques need less expertise for the detection of cryptic species within the bioindicator community. Although these methods require detailed knowledge of the molecular diversity within taxa in order to design efficient specific primers to avoid cross-reaction with non-target sequences. Based on rDNA (large subunit) LSU, RAPD and real-time PCR results and melting curve analysis, a model system to detect marine, planktonic, bloom -forming dinoflagellate *Prorocentrum minimum* using the DNA extracted from the Golden Horn Estuary bloom samples and mixed samples.
*Keywords: Bio-Indicators, Toxic Blooms, Population Dynamics, Phytoplankton, Bosphorus*

**Introduction**
DNA directly amplified from the environment have revealed a rich biodiversity of marine dinoflagellates that has escaped routine detection using microscopy [1]. The rDNA amplification approach to explore for the presence of unknown dinoflagellate DNA in the marine environment includes dinoflagellate-specific PCR of DNA extracted from field samples and cloning and sequence analysis of randomly selected clones [2]. DNA melting curve analysis is a technique that can be applied post-PCR to provide the melting temperature of the amplification products [3]. Sequence differences of each PCR amplicon can be distinguished by the melting curve that is affected by the GC content of the PCR product and the absolute order of the bases in the sequence [4, 5].

**Materials and Methods**
Field samples used in this work are morphologically identified dinoflagellate *Prorocentrum minimum* obtained from Golden Horn 26.07.2000 bloom. The number of cells were counted to be $70x10^6$/lt [6]. DNA isolation was done using MN Nucleospin Plant II kit. The sequences of the primers are given in Table 1. For real-time PCR Takara's SYBR Premix Ex Taq kit has been used. Real-time PCR has been done in Rotorgene Thermal Cycler. Confirmation of the real-time results were done on 2% Agarose gels (Figure 1). The size markers used in gel electophoresis have the band sizes 1000bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp and 100 bp in descending order.

**Results and Discussion**
Instabilities in the aquatic ecosystem should be monitored routinely. There are some bioindicator species that can be biomonitored for red-tide events in the Sea of Marmara. *Prorocentrum minimum* is a toxic species causing frequent red-tides especially in Haliç. In our study we have done the detection of this bioindicator species by using real-time PCR as a supporting tool for conventional methods of detection which will facilitate the routine monitoring of this species in the Sea of Marmara. Our approach, using the polymerase chain reaction (PCR) amplification of large subunit gene sequences (LSU rDNA), *P. minimum* specific sequences and RAPD and sybrgreen RAPD-real-time PCR is a useful and cost-effective genotyping method that can be used to determine both the population complexity and species identification of organisms in harmful algal blooms [7, 8]. DNA fragments (represented as bands on the gel) that were excised from agarose gel and were sequenced to determine the genetic variation data due to spontaneous mutations or taxonomic differences among the various bloom forming *Prorocentrum minimum* clones. There seems to be intra-specific variation, although spatial and temporal origins of the Haliç bloom samples was the same and approximately 100% cells as biomass and diversity belong to *P. minimum* [6]. Melting temperature differences are indicating this well and the xn PCR data confirmation with 2%agarose gel electrophoresis figures are indicating the variation as formed polymorphic bands and heteroduplexes detected and shown with arrows in figures confirmed with the sequencing data. x10 real-time LSU-rDNA amplification confirmation of Haliç 2000 *Prorocentrum minimum* bloom DNA indicating the dimorphism in the amplicon.

Tab. 1. Reproducible Tm peak ranges (C) after melting curve analysis for LSU rDNA and for species

| Name | Primer Sequence (5'→3') | Specification/Amplicon size | Reproducible Tm peak ranges (°C) after melting curve analysis |
|---|---|---|---|
| LSU-D1R | 5'-ACC CGC TGA ATT TAA GCA TA-3' | 650-850 bp | 87.0-87.7 |
| LSU-D2C | 5'- CCT TGG TCC GTG TTT CAA GA-3' | 650-850 bp | |
| Minimum F | 5'-GGG TCA TGG TAG CTC GTC TA-3' | 165 bp | 87.2-88.5 |
| Minimum R | 5'-CGT CTT TGT GTC AGG GAA AT-3' | 165 bp | |

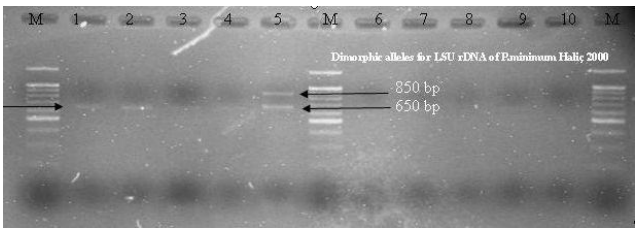


Fig. 1. X10 real-time LSU-rDNA amplification confirmation of Haliç 2000 *Prorocentrum minimum* bloom DNA indicating the dimorphism in the amplicon

## References
1 - Lopez-Garcia, P., Rodriguez-Valera, F., Pedros-Allo, C., Moreira, D., (2001). Unexpected diversityof small eukaryotes indeep-sea Antarctic plankton. Nature 409,603–607.
2 - Lakeman, M. B., P. von Dassow, et al. (2009). "The strain concept in phytoplankton ecology." Harmful Algae 8(5): 746-758.
3 - Robinson, B.S., Monis, P.T., Dobson, P.J., (2006). Rapid, sensitive, and discriminating identification of Naegleria spp. by real-time PCR and melting-curve analysis. Appl. Environ. Microbiol. 72, 5857–5863
4 - Monis, P.T., Giglio, S., (2006). Nucleic acid amplification-based techniques for pathogen detection and identification. Infect. Genet. Evol. 6, 2–12.
5 - Park, T., Park, G., Park, Y., Kang, Y., Bae, H., Kim, C., Jeong, H., Lee, Y., (2009) "Identification of the dinoflagellate community during Cochlodinium polykrikoides (Dinophyceae) blooms using amplified rDNA melting curve analysis and real-time PCR probes" Harmful Algae 8 430–440.
6 - Tas, S.,Yilmaz, I. N., Okus, E. (2009). Phytoplankton as an Indicator of Improving Water Quality in the Golden Horn. Estuary Estuaries and Coasts DOI 10.1007/s12237-009-9207-3.
7 - Sonnenberg, R., A. W. Nolte, et al. (2007). "An evaluation of LSU rDNA D1-D2 sequences for their use in species identification." Front Zool 4: 6.
8 - Scorzetti, G., L. E. Brand, et al. (2009). "Multiple simultaneous detection of Harmful Algal Blooms (HABs) through a high throughput bead array technology, with potential use in phytoplankton community analysis." Harmful Algae 8(2): 196-211.