High-throughput isolation of oyster DNA facilitates diagnosis of "Dermo"

Article · January 2000

CITATIONS
8

READS
161

3 authors:

José A. Fernández Robledo
Bigelow Laboratory for Ocean Sciences
79 PUBLICATIONS  1,316 CITATIONS

See Profile

Wolf T Pecher
University of Baltimore
17 PUBLICATIONS  112 CITATIONS

See Profile

Gerardo R Vasta
University of Maryland, Baltimore
220 PUBLICATIONS  6,701 CITATIONS

See Profile

Some of the authors of this publication are also working on these related projects:

Perkinsus Illustrations View project

All content following this page was uploaded by José A. Fernández Robledo on 26 June 2017.

The user has requested enhancement of the downloaded file. All in-text references underlined in blue are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.
High-throughput isolation of oyster DNA facilitates diagnosis of “Dermo” disease

The parasite Perkinsus marinus, the causative agent of “Dermo” disease, has been linked with mass mortalities of the eastern oyster Crassostrea virginica along the Atlantic and Gulf Coasts of the United States. Here we describe a method for efficient high-throughput DNA isolation from oysters using the QIAGEN® DNeasy 96 Tissue Kit, and a sensitive, species-specific, PCR-based method for the diagnosis of P. marinus.

José A. F. Robledo, Wolf T. Pecher, and Gerardo R. Vasta
Center of Marine Biotechnology, University of Maryland Biotechnology Institute, MD, USA

“Dermo” disease which has caused heavy mortalities in many oyster populations in the Northeastern United States, is caused by infection of oysters by the parasite Perkinsus marinus. Other Perkinsus species in Europe and Australasia have also been associated with disease in commercially relevant mollusk species including abalones, scallops, and clams. We have developed a sensitive, species-specific PCR-based assay for the detection of P. marinus, which can detect a single P. marinus cell spiked in approximately 30 mg of oyster tissue (1, 2). The non-transcribed spacer (NTS) of the rRNA gene cluster was selected as the target DNA sequence that would be unique to this parasite. The species specificity of the assay was confirmed by testing other known Perkinsus species. We have subsequently developed PCR assays for the detection of other Perkinsus species, including the European species P. atlanticus (3). In every case, the initial step in assay development was the selection and optimization of a rapid and reliable method for routine isolation of genomic DNA from bivalve tissues. Tissues were routinely processed using the QIAGEN DNeasy Tissue Kit. However, the increasing number of samples processed in our laboratory for experimental purposes, added to those received from oyster producers for certification of parasite-free oyster seed, required a higher throughput assay platform. This study was designed to test a 96-sample format for the DNA purification from bivalves using C. virginica/P. marinus as a model.

Materials and methods

Isolation of DNA

DNA isolation from Crassostrea virginica was based on the Mouse Tail Protocol in the DNeasy 96 Tissue Kit Handbook with modifications as follows. DNA was purified from various tissues including mantle, gills, and rectum (Figure 1). Tissue samples of 20, 40, and 60 mg were lysed overnight. No RNase A treatment was carried out in this particular experiment. Following the addition of Buffer AL/E, the samples containing the digested tissues were vigorously mixed, and loaded into the wells of a DNeasy 96 plate with the aid of an electronic multichannel pipette. The plate was centrifuged at 6000 rpm for 20 min. The membrane was washed and dried following the provided protocol and DNA was eluted with 100 µl of sterile water. Nucleic acid concentrations were determined by A260 measurements, and DNA quality was analyzed by agarose gel electrophoresis. DNA was also isolated from oyster hearts and cultured P. marinus using the DNeasy 96 Tissue Kit and the protocol described above.
### References


### PCR

PCR primers (5′-CAC TTG TAT TGT GAA GCA CCC-3′) and (5′-TTG GTG ACA TCT CCA AAT GAC-3′) that amplify a 307 bp target region within the NTS domain located between the 5S and SSU rRNA genes from *P. marinus* were used for detection of the parasite (1, 2). PCR assays contained 1 µg sample DNA in reaction buffer, 1 µM each of the PCR primers, 200 µM each dNTP, and 1.5 units of Taq DNA polymerase in a total volume of 25 µl. Positive control reactions contained 50 ng purified *P. marinus* DNA as a template. In negative controls template DNA was substituted by distilled water. PCR products were resolved in a 1.5% agarose gel in TAE buffer.

### Results

The use of the QIAGEN DNeasy 96 Tissue Kit and the modified procedure described above delivered high-quality genomic DNA in high yields, from oyster heart, mantle, gills, and rectum. For rectum and gill tissue, an increase of the sample size up to 60 mg resulted in a proportional increase of nucleic acid yield. This trend was less apparent when isolating DNA from mantle tissue, where the system probably became saturated (Figure 2). Although in tissue samples larger than 40 mg DNA yields were increased, a reduction of the $A_{260}/A_{280}$ ratio was observed (data not shown). Variability of yield was higher for DNA purified from larger amounts of tissue.

### Discussion

Most lifeforms inhabiting the oceans can be affected by disease. Recently, reports of mass mortalities due to disease outbreaks affecting marine organisms have been increasing (5). Both climate changes and human activities may have accelerated global transport of species, bringing together pathogens and previously unexposed host populations. Hence, in order to effectively monitor the health of the oceans, it is of paramount importance to develop novel diagnostic technologies, or to adapt methods developed for other systems, that can be easily implemented in the marine environment. The 96-well format method for the purification of DNA, in conjunction with the PCR reaction, currently enables us to obtain diagnostic results on 190 oyster samples within a single working day. Although the dissection of the bivalves is the lengthiest step in the diagnostic process, the standardized DNA extraction methodology in a 96-well format, has enabled us to significantly increase sample throughput. In addition...
to oysters, we have now applied the DNeasy Tissue Kit to DNA isolation from a variety of marine organisms, with minimal modifications (disruption of the cells with glass beads and increasing the amount of proteinase K and/or incubation time) to the supplied protocol. These organisms include clams, mussels, and most recently, marine phytoplankton (for detection of the toxic dinoflagellate *Pfiesteria piscicida*). Furthermore, because “Dermo” disease has been reported in oyster beds since the early 1950’s [6], the testing of archival samples is critical for the understanding of how outbreaks have spread. We have recently obtained promising results using the DNeasy Tissue Kit for purification of DNA from fixed and paraffin-embedded oyster tissue samples, and plans for the near future include a thorough study of archival sample collections at several institutions.

**Acknowledgements**

This study was supported by supported by DOC Cooperative Agreements No. NA47FL0163 and No. NA57FL0039 awarded by NOAA, Oyster Disease Research Program, Sea Grant College and Grant No. NA00AADSG063 awarded by NOAA through the Maryland Sea Grant to G. R. V.

---

**Table 1. List of host organisms and target species from which genomic DNA is routinely isolated in our lab using the DNeasy Tissue system.**

<table>
<thead>
<tr>
<th>Host organism</th>
<th>Target species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine phytoplankton</td>
<td><em>Pfiesteria piscicida</em></td>
</tr>
<tr>
<td>Eastern oyster (<em>Crassostrea virginica)</em></td>
<td><em>Perkinsus marinus</em></td>
</tr>
<tr>
<td>European flat oyster (<em>Ostrea edulis</em>)</td>
<td><em>Bonamia ostreae</em></td>
</tr>
<tr>
<td>Australian blood cockle (<em>Andara trapezia</em>)</td>
<td><em>Perkinsus sp.</em></td>
</tr>
<tr>
<td>Baltic clam (<em>Macoma balthica</em>)</td>
<td><em>Perkinsus sp.</em></td>
</tr>
<tr>
<td>Carpet shell clam (<em>Ruditapes decussatus</em>)</td>
<td><em>Perkinsus atlanticus</em></td>
</tr>
<tr>
<td>Hardshell clam (<em>Mercenaria mercenaria</em>)</td>
<td><em>Perkinsus sp.</em></td>
</tr>
</tbody>
</table>

---

**Ordering Information**

<table>
<thead>
<tr>
<th>Product</th>
<th>Contents</th>
<th>Cat. No.</th>
<th>Price (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNeasy 96 Tissue Kit (4)*†</td>
<td>For 4 x 96 DNA preps: 4 DNeasy 96 plates, Proteinase K, Buffers, Square-Well blocks, Tape Pads, Collection Microtubes (1.2 ml), Caps</td>
<td>69581</td>
<td>785.00</td>
</tr>
<tr>
<td>DNeasy Tissue Kit (50)*</td>
<td>50 DNeasy Spin Columns, Proteinase K, Buffers, Square-Well blocks, Tape Pads, Collection Tubes (2 ml)</td>
<td>69504</td>
<td>92.00</td>
</tr>
<tr>
<td>Centrifuge 4-15C (120-V)</td>
<td>Universal laboratory centrifuge with brushless motor (120 V, 60 Hz)</td>
<td>81010</td>
<td>7,995.00</td>
</tr>
<tr>
<td>Plate rotor 2 x 96</td>
<td>Rotor for 2 QIAGEN 96-well plates for use with QIAGEN Centrifuges‡</td>
<td>81031</td>
<td>1,759.00</td>
</tr>
</tbody>
</table>

* Other kit sizes available; please inquire.
† Requires use of the QIAGEN 96-Well-Plate Centrifugation System.
‡ The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuges 4-15C, and 4K15C from QIAGEN, and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH.

---


