

# Small subunit rRNA gene sequence of *Crassostrea virginica* (Gmelin) and a comparison with similar sequences from other bivalve molluscs

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As a preliminary measure in developing species-specific nucleic acid probes for commercially important shellfish and their protozoan parasites, we have cloned and sequenced the ribosomal RNA gene from blood and sperm of the eastern oyster, *Crassostrea virginica* (Gmelin). DNA was extracted as in (1) and rDNA was amplified by PCR using eukaryote specific oligodeoxynucleotides complementary to conserved regions at the 5' and 3' termini of the coding region for the 18S-like rRNA gene (2, 3). Amplified rDNA was cloned into pGEM-11zf(-) and the complete nucleotide sequence of 1816 bp was determined from each strand by the dideoxy chain termination method employing other eukaryote and universal rRNA oligodeoxynucleotide primers.

Phylogenetic analysis (using PAUP, 4) with this and four other partial (5, 6) and complete (7) bivalve mollusc rDNA sequences (*Spisula solidissima*, *Mya arenaria*, *Mytilus edulis* and *Placopecten magellanicus*), with the nudibranch *Anisodoris nobilis* as an outgroup, supports a phylogeny derived from morphological characters (8); i.e. (*Anisodoris*, (*Crassostrea*, *Placopecten*, (*Spisula*, *Mya*), *Mytilus*)). The only two complete sequences, *Crassostrea* and *Placopecten*, which are taxonomically the most closely related, shared a 91.4% sequence homology.

Techniques involving the identification and quantification of larval marine molluscs are becoming increasingly important in marine ecology, fisheries and aquaculture, and to date have relied heavily on morphometric measurements which are often inadequate (9) particularly within genera. Alignment of the rDNA sequence data of each of the above mentioned commercially valuable bivalves, has allowed us to design putative probes for their accurate identification. The following probe sequences are derived from variable regions of our alignment. They are designed to hybridize against variable region V2 in the eukaryote-specific helix E10-1 (10) of the rRNA molecule, and satisfy criteria outlined in (11):

5'-AAACGGACGGCCTTGCGA *Crassostrea virginica*  
 5'-AGAGTGACGGCCTTTGCGA *Placopecten magellanicus*  
 5'-TCTACCAATCCGACGGG *Mytilus edulis*  
 5'-TTCGGAGTACCACGTTGGG *Spisula solidissima*  
 5'-CCCACAGAGCGACGCAT *Mya arenaria*

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