Proposal for Construction of BAC Libraries from the Eastern Oyster, *Crassostrea virginica* and the Pacific Oyster *C. gigas*.

The Oyster Genome Consortium[1].

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Importance of the oyster to biological and biomedical research.

Two important species of oyster occur in US coastal waters, the Pacific Oyster (*Crassostrea gigas*) and the Eastern Oyster, (*C. virginica*). Both species are the subject of considerable research activity in the US, Europe and Japan. While oysters are, on a mass basis, the most heavily aquacultured organisms in the world (FAO Fisheries Department Summary Tables of Fishery Statistics http://www.fao.org/fi/statist/summtab/default.asp), the two considerations that underlie this request are not commercial, but relate to fundamental issues of evolution and the role that oysters play with respect to the marine environment and human health.

Evolution:

Bilateral symmetry is found in three major clades of animals: the deuterostomes (which includes the vertebrates); the ecdysozoa (which includes the arthropods and nematodes); and the lophotrochozoa (which includes the molluscs and annelids). The first two clades are well represented with genomically-enabled model species. For deuterostomes this list includes *Amphioxus, Oikopleura, Ciona spp*., *Fugu*, zebrafish, *Xenopus*, sea urchin, chicken, mouse and man. In the case of the ecdysozoa, the list includes *Drosophila, Anopheles,* the honeybee and *Caenorhabditis elegans*. Lophotrochozoan species are studied by investigators interested in questions such as 1) Embryonic development and the phylogenetic diversity of the body plan (Nederbragt et al., 2002; O'Brien and Degnan, 2002; Korakis and Martindale, 2001). 2) The evolution of molecular mechanisms of immune recognition and response to stress (Jenny et al., 2002; Tanguy and Moraga, 2001; Tanguy et al., 2001; Gueguen *et al* 2003, http://www.ifremer.fr/GigasBase/). The progress of these and other research projects would benefit enormously from the wide availability of genomic tools, including BAC libraries. In order to achieve an integrated understanding of the genetic basis for the diversity of animal life forms, a detailed understanding of representative lophotrochozoan species will be necessary. Major Classes within the phylum Mollusca include the Cephalopoda (octopus, squid), Gastropoda (snails); and bivalves (oysters, clams). Only a single lophotrochozoan species (*Biomphalaria glabrata*, a gastropod mollusc) is the current subject of BAC library construction.

The marine environment and human health.

The coastal zone of the US is home to a significant and rising proportion of the population, and the pressures of residential, industrial and tourism-related development have resulted in degradation of the coastal marine environment. Numerous threats to human health arise from the marine environment, including infectious diseases and harmful algal blooms (Committee on the Ocean's Role in Human Health, 1999). Infectious agents include viruses such as hepatitis A, and the caliciviruses including the Norwalk virus, and bacteria such as *Vibrio parahemolyticus, V. vulnificus, Escherichia coli, Salmonella spp* and *Shigella spp*. Harmful algae such as *Pfiesteria spp*. and *Karenia spp*. produce highly toxic environmental chemicals. Infectious agents and harmful algae are concentrated by filter-feeding shellfish, and the consumption of shellfish, including oysters, is thus an important mode of transmission of infectious disease, as well as Paralytic Shellfish Poisoning, Amnesic Shellfish Poisoning, Neurotoxic

Shellfish Poisoning, and Diarrhetic Shellfish Poisoning (Committee on the Ocean's Role in Human Health, 1999). Oysters (and other molluscs implicated in both infectious disease and shellfish poisoning) are not just passive vectors of the agents that they accumulate while feeding. Their relationship to these organisms is dynamic and intimately connected with the overall health of the marine coastal environment. The role of oysters in filtering coastal waters has been dramatically illustrated by the decline of the Chesapeake Bay, of which a primary proximal cause has been the loss of most of the oyster population. This loss has been caused by a mixture of factors including anthropogenic contamination of the Bay and commercial overharvesting, but physical factors (low rainfall causing increased salinity) and disease (parasitic infestation of oysters with *Perkinsus marinus* and *Haplosporidium nelsoni*) have also played a role (cf Bobo *et al*. 1996). There is good reason to believe that oysters may, through their role as filter feeders, help control the nature of the algal populations of the coastal estuarine waters (Wetz et al., 2002). The relationship between oysters, their physical environment, human pathogens, oyster pathogens, and the ecology of the coastal environment is very complex, and the dissection of this relationship is being undertaken by many investigators using not only traditional cellular and biochemical studies, but also functional genomic approaches. It should be mentioned that oysters are also highly efficient bioaccumulators of toxic heavy metals such as lead and cadmium, which can lead to poisoning of humans who consume oysters from contaminated sites. Although this threat to human health has been substantially reduced by environmental standards in most developed nations, the genetics and biochemistry of oyster uptake, and response to, heavy metals exposure is an active area of research (e.g. Tanguy and Moraga, 2001; Tanguy et al., 2001).

Uses for the Oyster BAC libraries:

The requested BAC libraries will provide a major genomic resource that will benefit several research communities, as described below.

Comparative Genomics:

Low-density linkage maps have been developed for both Pacific and Eastern Oysters (presented by D. Hedgecock and X. Guo at the Plant, Animal Genome XI Conference, San Diego, 2003). Complete fingerprinting of the oyster BACs to generate physical maps of the oyster genomes, and the fusion of these physical maps with high-density genetic maps are a long-term goal of the Consortium. However, the availability of BAC libraries will permit the immediate initiation of small-scale physical mapping. Selected BAC clone inserts will be mapped, by fluorescent *in-situ* hybridization (FISH), to chromosomes (Labs of Guo, Gaffney, Hedgecock, Reece). Genes of particular interest that have been identified from the growing collection of oyster cDNAs (Jenny et al., 2002; Gueguen *et al* 2003. http://www.ifremer.fr/GigasBase/) will be mapped, by filter hybridization, to BAC clones (groups of Vasta, Warr, Chapman, Gomez-Chiarri, Reece) and, by FISH, to oyster chromosomes

***Evolution*:**

The genetic basis of evolutionary change, particularly with regard to speciation, remains one of the least understood phenomena in biological science. While considerable data exist concerning changes in nucleotides, amino acid composition of structural genes, gene arrangements, etc., the roles these changes play in speciation are not well understood. In addition, the dynamic interactions of genes (epistasis) has long been thought to be an important component of adaptation (Wright, 1931), and it is only with the development of genome-enabled science that this issue has become addressable. Combining information on regulatory regions (derived from BAC libraries) with studies of gene expression profiles is an obvious mechanism to study speciation. The current application will provide the means to accomplish this end in two closely related species. The fact that these species are known to differ in a variety of physiological and immunological characteristics, provides an important link to genetic adaptation. By extension, once candidate genes and the dynamics of their expression are identified, then investigations can be extended to other *Crassostrea* species and perhaps other oyster genera as well.

Structure and Expression of Oyster Genes - Comparative Physiology and Immunology:
Investigators have, through cDNA and limited genomic cloning, been studying several aspects of oyster molecular genetics related to comparative immunology, and the physiology of the response to heavy metals and infection. There are too many references to permit a comprehensive survey in this proposal, but typical investigations include the response of metallothionein genes to heavy metals (Tanguy and Moraga, 2001; Tanguy et al., 2001), and the evolution of immune recognition and response pathways (Escoubas et al., 1999; Montagnani et al., 2001; Jenny et al., 2002; Gueguen *et al*. 2003 http://www.ifremer.fr/GigasBase/). Recent initiatives from several laboratories are aimed at understanding the relationship between the oyster and the parasite *Perkinsus*. The availability of BAC libraries would facilitate ongoing molecular genetic projects such as these, and would also attract many laboratories already conducting cellular and biochemical research in oysters to adopt molecular genetic approaches.

Justification for requesting BAC libraries from 2 species of Oyster:
The Pacific and Eastern Oyster are related species, separated by 10 million years of evolution (Jozefowicz and Foighil,1998), and as such a comparison of their chromosomal structure will give insight into genomic evolution in the bivalve molluscs, as discussed above. However, these 2 species are known to differ in important biological respects that are not yet understood, but clearly have a genetic basis. One such major difference relates to their susceptibility to *P. marinus*, the causative agent of Dermo disease, which is responsible for the significant decline in oyster populations of both the East and Gulf Coast regions. It is not an exaggeration to voice concern that the spread of this parasitic disease will effectively destroy the Eastern Oyster population. Of great interest in this context is the observation that the Pacific Oyster is tolerant to experimental challenge with *P. marinus* (Meyers et al., 1991; Barber and Mann, 1994; Calvo et al., 1999). Several groups are pursuing elucidation of the genetic basis for this difference, and success will provide insight into fundamental, and most likely novel, mechanisms of innate immunity in molluscs.

The Oyster Research Community:
The Oyster Genome Consortium, an informal group, consists of US-based scientists interested in oyster research at a genetic/genomic level, and there is a significant and similarly sized community of oyster researchers in Europe and Japan. As the field is stimulated by the availability of genomic tools, the number of investigators would be expected to increase well beyond its current level

BAC based genomic sequencing of the Oyster:
To the best of our knowledge the oyster has not been proposed as a subject for whole-genome sequencing.

Other Available Oyster Genomic Resources:
The Marine Genome Group in Charleston is building, in an ongoing effort, an EST database for *C. virginica* (see e.g. Jenny et al., 2002) and has the capacity to produce microarrays for interested investigators once the size of the unigene collection has reached an appropriate size (3,000 unigenes is the initial target). The French IFREMER Group based in Marseilles is undertaking a similar exercise for *C. gigas* (Gueguen *et al*. 2003) and sequence data, clones and, eventually, microarrays will also be available from this source. The current genetic maps are based primarily on polymorphic microsatellite markers, and several laboratories are now cataloguing SNP markers in oyster genes. Massively parallel signature sequencing of more than 3.1 million cDNAs has been carried out on two inbred and two hybrid populations of larval Pacific oysters by Lynx Therapeutics (Hedgecock et al. 2002). Statistical analysis of the variation in expression levels among these four populations has identified about 150 candidate genes likely to play a role in the dramatic growth heterosis observed in Pacific oysters (Hedgecock et al. 1995).

Strain of Oyster Proposed:
We will use the 51x35 hybrid of *C. gigas*, for which we have a complete pedigree back to Dabob Bay naturally-spawned founders and replicated growth data (at experimental and commercial scales of culture and demonstrating heterosis). A heterosis QTL mapping study is in progress, and it is these lines from which the Lynx expression library and analysis have been developed. A comparable line is not available for *C. virginica* and we will select a representative wild caught individual for BAC construction.

Size of the Oyster Genome:
The oyster genome is compact: the haploid chromosome number of both *C. gigas* and *C. virginica* is 10, and the estimates of the size of the haploid genome range from 700 Mb for *C. virginica* to 900 Mb for *C. gigas* (Gregory, 2001).

Availability of Oyster Genomic DNA:
The proposed source for oyster genomic DNA is sperm. This can be harvested in large quantities (exceeding 10^9 cells) from an individual. Sperm are only available during the breeding season (typically beginning in May-June, but somewhat earlier in the southern part of the range) and BAC-ready genomic DNA has not yet been prepared. The Marine Genomics Group in Charleston has visited the Clemson University Genome Center to learn the techniques for agarose-plug embedding of cells, and the *in situ* digestion method to produce high molecular weight "big" DNA. This technique was successfully applied to preparations of sperm from another marine invertebrate (the Pacific White Shrimp) and we feel confident in our ability to produce BAC-library quality DNA from oyster sperm.

Specifications of the libraries:
A standard BAC library vector such as pBeloBAC, with insert sizes ranging from 100 – 150 kbp would be appropriate. Deep coverage libraries (7-10x) would be highly advantageous, permitting a reasonable degree of confidence that essentially all of the organism's genes were represented in the library.

Time Frame:
The oyster research community would be able to utilize a BAC resource as soon as it was available, both for adding physical mapping capacity to the existing genetic maps, and to clone genes of interest (e.g. metallothioneins) to study their structure.

Other Support Requested:
None to our knowledge.

Existing BAC libraries:
To the best of our knowledge, no BAC library for *C. virginica* exists. A BAC library of *C. gigas* has been created by Shimizu (Tokyo) but is not available to other researchers.

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