Genetic Marker Discovery for Gene Map Construction in the Pacific White Shrimp (*Litopenaeus vannamei*)

A.S. Leaflet R2427

Zhi-Qiang Du, postdoctoral research associate;
Danielle Gorbach, graduate student;
Max F. Rothschild, professor, Department of Animal Science; Guillermo Jaramillo, Shrimp Improvement Systems

Summary and Implications

The Pacific White Shrimp (*Litopenaeus vannamei*) is important to the aquaculture industry and as a food source, but only limited genome information exists. The demand for specific shrimp broodstocks with high disease resistance and growth rate is increasing so the construction of a genetic map with easily usable genetic markers is a high priority. Using all the available public genomics information, a large-scale discovery effort for single nucleotide polymorphism (SNP) genetic markers has produced the identification of 1,576 SNPs in 545 unique contigs. The genotyping work will be performed on the standardized Sequenom™ platform on a ready-to-use shrimp resource family, which will be used to develop a shrimp genetic map, as well as facilitate the mapping of quantitative trait locus (QTL) for growth rate and disease resistance.

Introduction

The shrimp industry is expanding annually; however, unstable production does occur due to environmental problems and disease challenges. This makes the selective breeding of shrimp strains which are resistant to pathogens and have a high growth rate a high priority. Discovery of large number of genetic markers and the construction of genetic map with high density coverage of the whole shrimp genome would be of great value for future breeding programs. Unfortunately, there is very limited genome sequence information available in nearly all shrimp species. Recently, we witnessed the fast development of next-generation technology for low cost and high throughput sequencing and genotyping using single nucleotide polymorphisms (SNPs) to search for the molecular mechanisms underlying important phenotypes. In this process of the large-scale discovery of SNP markers, we combined bioinformatics and molecular technologies and tools, to mine and annotate all the available public data relative to the Pacific White Shrimp, *Litopenaeus vannamei*.

Materials and Methods

A shrimp resource family was created using a standard 3 generation F2-design. From the parental F1 generation, 16 animals were selected for SNP detection. Diverse data resources have been used for mining SNPs, including the public databases (marine genomics database and NCBI) and other sequencing data. All the sequence data were clustered, and input into a newly designed computational pipeline (SNPidentifier) programmed in the computer language called Perl to detect SNPs. PCR Primers were designed for all the sequences containing predicted SNPs using Primer3, and PCR products were sequenced. Sequencing results were clustered and checked manually by Sequencher™ 4.8 (Gene Codes Corporation). Some of the SNPs were directly tested by the restriction fragment length polymorphism (RFLP) method with appropriate restriction enzymes. The Sequenom MassArray iPLEX™ platform has already been standardized and will be used to carry out the large-scale SNP genotyping work. After quality control, the genotyping data will be obtained and be used to construct a genetic map using CRIMAP.

Results and Discussion

Currently, a total of 1,576 SNPs in 545 unique genomic contigs have been discovered. From the public data source, the 25,937 *L. vannamei* ESTs were clustered into 3,532 contigs, in which 205 contigs contributed 768 SNPs. From another public dataset, we found 564 SNPs in 250 contigs. Furthermore, we found 74 SNPs distributed in 34 short tandem repeats, 170 SNPs in 56 candidate genes with full-length cDNA sequences. SNPs identified in the short tandem repeats could also help the final integration of our genetic map into a common framework. Large-scale genotyping work is ongoing now on the Sequenom platform. A systematic strategy is vital to the success of genetic map construction, especially when inundated with large amounts of genomics information, rapidly available with the help of next-generation sequencing and genotyping technologies. Furthermore, SNPs discovered can be used in the tracking and identification of genetic factors involved in disease resistance and other economically important traits.

Acknowledgments

We appreciate the funding from CP Indonesia, Shrimp Improvement Systems, Gold Dragon Research and State of Iowa and Hatch Funding. Technical help from Dr. Rothschild's lab group and that of Dr. Patrick Schnable is appreciated.