

Harnessing the NGS-Based RNA Sequencing Technology for Genetic Resource Identification and Applications in Bivalve Shellfish Aquaculture

Abbreviations: FAO: Food and Agriculture Organization of the United Nations; OGC: Oyster Genome Consortium; NGS: Next-Generation Sequencing; SSH: Suppression Subtractive Hybridization; SSR: Simple Sequence Repeat

Editorial

Aquaculture is the fastest growing animal-food producing sector and promises an important role in meeting the food-demand of the global population. Aquaculture supports the livelihoods of 10-12% of world's population with sustainable economic opportunities. The sector has generated global recognition and is included in FAO's (Food and Agriculture Organization of the United Nations) strategic objectives of alleviating malnutrition and hunger, ushering an era of food security. There is a strong undercurrent of entrepreneurship activities in the sector, which has satiated the demand for fish globally, and has been efficient in reducing rural poverty and community resilience to climate change crisis. In 2010, the global fish production by capture and aquaculture was 148 million tons and in 2012, while global capture fishery production was stable, global aquaculture production showed a growth of more than 90 million tonnes [1]. The world bivalve mollusc production that represents 10% of total world aquaculture production has increased substantially to about 14.6 million tons in 2010. The major share of bivalve production with 10.35 million tons in 2010 comes from China, followed by Japan (819,131 tons), the USA (676,755 tons), and the Republic of Korea (418,608 tons).

With a growing incentive in bivalve aquaculture and fishery production, technological developments such as polyploidy production, ongoing selection and breeding programs have flourished [2]. Scallops, oysters, clams, and mussels are among the important bivalve species in global aquaculture. In the bivalve trade, scallops are the most important species with 46% of value, closely followed by mussels (26%). Besides value in aquaculture, bivalves are organisms of choice in environmental monitoring studies, as they are adapted to a variety of environments, and due to their filter feeding habits, can accumulate xenobiotic compounds.

In response to the importance of bivalves to global aquaculture production and environmental monitoring studies, bivalve genomics made a head start with the Oyster Genome Consortium (OGC), comprising of 70 individuals from 11 countries [3]. Under this community genome initiative, the Pacific oyster (*Crassostrea gigas*) was a candidate for genome sequencing that provided unparalleled resources for a broad range of studies on evolution and environmental sustainability. The study was one of the initial attempts to harness next-generation sequencing (NGS) technology for understanding the complexity of bivalve genomes. Parallel to

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the use of NGS in bivalve genomics, the high-throughput, NGS of RNA, called RNA-seq has been useful in rapid dissemination of important genomic information in bivalves, especially the identification of transcripts for adaptation and reproduction with the development of genetic markers. RNA-seq has been an attractive option for studying non-model organisms as it is more reliable and cheaper than building genomes.

The NGS platforms for whole-transcriptome and tissue-specific transcriptome characterization of bivalve species includes the 454/Roche and Solexa/Illumina. Most NGS *de novo* transcriptome sequencing projects in bivalves have used the 454/Roche pyrosequencing system. Roche's 454 GS-FLX platform provides a longer read size with deep sequencing coverage and has been widely used for *de novo* transcriptome sequencing of bivalves such as *Bathymodiolus azoricus* [4], *Ruditapes philippinarum* [5], *Mytilus edulis* [6], *Sinonovacula constricta* [7] and *Crassostrea hongkongensis* [8]. Lately, Illumina sequencing technology (short, paired-end reads) has been the preferred choice due to algorithmic advances, highly replicable, and cost-efficient process for transcriptome characterization. In addition, it is a superior method for the identification of differentially expressed transcripts. The bivalve species for which Illumina facility was successfully used for transcriptome characterization includes *Haliotis midae* [9], *Chlamys farreri* [10], *Pecten maximus* [11], and *Crassostrea virginica* [12]. Notwithstanding the NGS platform used, genomic resources in the form of a rich repertoire of genes related to innate immunity, digestion, sex-determination, reproduction and environmental stress have been divulged from the analysis. We have also conducted the transcriptome characterization of an endangered freshwater pearl bivalve, *Cristaria plicata*, and have discovered a rich set of immune and reproduction-related genes. Additionally, we have characterized the simple sequence repeat

(SSR) markers that would be relevant for genetic characterization and conservation studies.

Conclusion

Although initial transcriptomic analysis in bivalves were based on technologies such as cDNA libraries, suppression subtractive hybridization libraries (SSH) and microarrays, RNA-seq provides high-throughput, unparalleled and unbiased gene expression information. The NGS platforms have provided rich resources for many economic bivalve species especially with reference to expression of genes related to vital metabolic, cellular, and immune processes. This has added to the meaningful genetic resource data stored in databases to be utilized as a public information platform for exploitation in functional analysis. In the near future, we can expect an increase in transcriptome sequencing that will provide clues for their potential applications in biomonitoring of environmental contaminants and further improve their accessibility for aquaculture production.

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