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[REVIEW]

The Importance of Total Genome Databases in Research on Akoya Pearl Oyster

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Pearl is the most valuable form of gem made by organisms and its beauty has been considered attractive and precious by people all over the world. Pearl oyster aquaculture system, in which a graft from the mantle pallium is transplanted with a nucleus into mother pearl oysters, is currently conducted at the industrial level. However, it is unclear what molecular mechanisms are involved in this system of pearl formation, which produces brilliant and beautiful nacreous layers. Since it takes long time to look for the proteins and genes possibly participating in the molluskan shell formation, another sophisticated strategy has been expected to screen candidate genes. One breakthrough in the latter area was the development of next-generation sequencing (NGS) in place of the automated Sanger method, the first generation sequencing technique. Using NGS, expressed sequence tag (EST) libraries were constructed from pallial mantle and pearl sac, which form the nacreous layer, and from the mantle edge, which forms the prismatic layer in the Akoya pearl oyster *Pinctada fucata*. Subsequently, the total genome sequence of the Akoya pearl oyster was analyzed. This database, together with the EST database mentioned above, is useful for identifying genes responsible for superior phenotype characters for pearl oyster aquaculture such as fast growth, disease resistance, easy domestication, and ability to produce high quality pearls. Subsequent marker-assisted breeding of the Akoya pearl oyster should establish strains with superior genotypes, producing even higher quality Akoya pearls.

Key words: biomineralization, genes, genome, mollusk, pearl oyster, shell formation

Pearls have attracting human attention all around the world since the prehistoric era. Up to the present, Japan has been a leading country for the production of pearls by farming of the Akoya pearl oyster, *Pinctada fucata*. The pearl is only one genuine gemstone that can be created artificially by using biological functions of mollusks that form the pearl sac, where the layers of bicarbonate crystals and organic substances are constructed in the acceptor step by step on the small nucleus, yielding aragonite crystals named nacre. The present-day round pearl produced in Akoya pearl oyster artificially was first industrialized in late 1890s in Japan by Mr. K. Mikimoto. To cope with industrial demands, the mechanisms involved in the nacre layer formation had been extensively studied mostly using biological and chemical approaches.

Biomineralized molluskan shells are formed by mineral precursor ions such as calcium, bicarbonate, and minor and trace elements, together with extracellular macromolecules such as proteins, glycoproteins and polysaccharides, yielding nacreous layers with aragonite crystals and prismatic layers with calcite crystals, the two types of the layer being formed in the inner and outer parts of molluscan shells, respectively. It has been suggested that shell formation is controlled by the successive deposition of mineralized and organic layers to form organic matrices, and thus the proteins concerned have been extracted from shells and characterized extensively to understand the mechanisms underlying the shell formation. One breakthrough in such protein chemical approaches was the determination of the complete primary structure of nacrein from the nacreous layers by Miyamoto and his colleagues in 1996 using molecular biological techniques including cDNA cloning (Miyamoto et al., 1996). Since then, various proteins responsible for nacreous layer formation have been isolated and characterized.

As it takes long time to look for the proteins and genes possibly participating in the molluscan shell formation, another sophisticated strategy was expected to be screening for candidate genes. In addition to protein chemistry and conventional cDNA cloning, larger screens for shell proteins have been conducted using advanced molecular biological techniques, such as differential display and suppression substractive hybridization to construct expressed sequence tag (EST) libraries, using mostly Sanger sequencing (e.g., Wang et al., 2012).

The automated Sanger method using capillary electrophoresis with fluorescence labeled nucleotides had dominated the industry for almost two decades for DNA sequenc-

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ing. Despite numerous technical improvements during this era, the low throughput and high costs of Sanger sequencing highlighted the need for new, improved technologies for sequencing genomes of large size. If we consider the automated Sanger method as the first-generation sequencing approach, the newer methods can be referred to as next-generation sequencing (NGS). Many NGS platforms have been established, such as the Roche 454 GS FLX system using pyrosequencing technology (Margulies et al., 2005), the ABI SOLiD system adopting the technology of two-base sequencing based on ligation sequencing (Mardis, 2008), the Illumina GA/Hiseq system using synthesis technology (Mardis, 2008), and the newer Ion Torrent PGM system based on the use of semi-conductor sequencing technology (Flusberg et al., 2010). NGS offers a major advance to produce an enormous volume of data cheaply with high efficiency.

Despite such progress, following data analyses and functional annotations were still challenges. Most sequencing projects used reads primarily or exclusively from Illumina sequencers, with read lengths ranging from 35 to 101 bp and with coverage depth ranging from 50- to 100-fold. These massive short sequences must be firstly *de novo* assembled prior to the downstream genome analyses. Subsequently, many excellent genome assemblers, such as SOAPdenovo and Velvet, which run large, whole-genome assemblies using short-read data, have been developed (Li et al., 2010; Zerbino et al., 2010). It is certain that today NGS technologies have taken precedence over the traditional Sanger method, and they are increasingly employed for molecular biological studies.

Kinoshita et al. (2011) employed the GS FLX 454 system and constructed transcriptome data sets from pallial mantle and pearl sac, which form the nacreous layer, and from the mantle edge, which forms the prismatic layer in the Akoya pearl oyster. They sequenced 260477 reads and obtained 29682 unique sequences. The group also screened novel nacreous and prismatic gene candidates by a combined analysis of sequence and expression data sets, and identified various genes encoding lectin, protease, protease inhibitors, lysine-rich matrix protein, and secreting calcium-binding proteins. They also examined the expression of known nacreous and prismatic genes in the EST library and identified novel isoforms with tissue-specific expressions. Soon after this publication, a number of other reports appeared, all devoted to comprehensive transcriptome analysis.

Last year, Sato and his colleagues reported the total genome sequence of the Akoya pearl oyster, using Roche 454 GS-FLX and Illumina Genome Analyzer IIx (GAIIx) instruments (Takeuchi et al., 2012). Its approximately 1150 Mbp genome at this draft stage provided ~12,000 gene models. This database also provided the first opportunity to closely study a bivalve mollusk genome. Moreover, this genome database provided a basic platform for further studies of biosynthesis of pearl, which has a significant importance in fisheries industry. This database together with EST database mentioned above is useful for identifying genes responsible for superior phenotype characters for pearl oyster aquaculture such as fast growth, disease resistance, easy domestication, and ability to produce high quality pearls, possibly employing DNA microarray analysis. Subsequent marker-assisted breeding of the Akoya pearl oyster should establish strains with superior genotypes, producing even more excellent Akoya pearls.

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