

## Use of RNA-seq in Aquaculture Research

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High throughput, next-generation sequencing techniques have been widely used for gene expression profiling and the study of signal transduction pathways due to their superior advantages over microarray technology, which requires previous genomic sequence or expressed sequence tag information [1]. Whole-transcriptome shotgun sequencing is known as RNA sequencing (RNA-seq) [2], which is a technology that employed the capabilities of next-generation sequencing to reveal a snapshot of the presence and quantity of transcripts in a transcriptome at a given time [3]. RNA-seq can help to capture and annotate the transcriptome [4], and to discover novel transcribed regions in the genomes of non-model aquatic animals [4-6]. It has also been proven to be a sufficient tool to capture the genes and pathways involved in many biological processes of aquatic animals [7-9]. Therefore, the use of RNA-seq has attracted the attention of aquaculture researchers in many areas of research, and successful example studies have been reported in many economical aquaculture species [10-13].

### Selective Breeding and Resource Conservation

RNA-seq studies are mainly focusing on SNP discovery as an effective tool. Studies have been done in channel catfish and blue catfish [14], common carp [15], and rainbow trout [12]. For instance, growth-rate related SNP markers in rainbow trout were identified by RNA-seq, which proved that RNA-seq is a fast and effective means for identifying SNPs, and can be used for marker development in non-model species lacking complete and well-annotated genome reference sequences [12]. RNA-seq based approach was used to develop molecular resources for *Villosa lienosa*, [13]. And in the study, 23,742 unigene were captured by BLAST against the National Center for Biotechnology Information non-redundant database and 36,582 microsatellites with sufficient flanking sequence for primer designing were identified for *V. lienosa*, indicating that RNA-seq is a powerful tool for rapid development of molecular resources in non-model species too.

### Disease Resistance and Immunology

The main use of RNA-seq in economical aquaculture species are focusing on finding the immune related genes or pathways by comparison of the whole transcriptome following pathogen challenge [8,11,16,17], and clarifying the host immune mechanisms underlying vaccine protection [18,19]. For instance, RNA-seq analysis of mucosal immune responses revealed signatures of intestinal barrier disruption and pathogen entry following *Edwardsiella ictaluri* infection in channel catfish [11]. 454 pyrosequencing-based RNA-Seq results revealed that apoptosis, mitogen-activated protein kinase signaling, toll-like receptor signaling, Wnt signaling and antigen processing and presentation pathways functioned importantly in defending against White Spot Syndrome Virus in white shrimp [10]. Similar studies were conducted in *Vibrio harveyi* challenged Asian seabass [8] and Japanese sea bass [17], white shrimp with Taura syndrome virus [20], and Chinese shrimp challenged with White Spot Syndrome Virus [21]. In zebrafish, RNA-seq was utilized to investigate the expression patterns of immunization-related genes immunized with vaccines against *E. tarda* [19], as well as in European sea bass with vaccines against *V. anguillarum* [18].

### Stress Physiology and Toxicology

To understand the complex molecular biological process of stress physiology or toxicology at whole transcriptome level, RNA-seq would be a practical and efficient technology to obtain the overall and relatively complete genes and pathways involved into the corresponding physiological response. RNA-seq showed that 604 genes were involved in heat stress-response pathways in *V. lienosa* [13]. Similar work was also conducted to determine the heat stress-induced gene expression profile in channel catfish [22], zebrafish [23] and rainbow trout [7]. RNA-seq was also employed to understand the mechanism of osmoregulation in Asian seabass [8], Amur ide [9], and Chinese mitten crab [24]. For instance, protein ubiquitination, ubiquinone biosynthesis, oxidative phosphorylation, mitochondrial dysfunction EIF2 signaling, IGF-1 signaling, and amino acid metabolism were found to be the top stress-related pathways in the Chinese mitten crab after ambient salinity challenge revealed by RNA-seq [24]. Similar studies were reported in white shrimp exposed to nitrite [25]. For the use of RNA-seq in aquatic toxicology, the transcriptomic response to polychlorinated biphenyl (PCB) exposure in embryos and larvae of Atlantic killifish was studied [26]. Similarly, the toxicological effects of perfluorooctane sulfonate on *Oryzias melastigma* embryos were detected by RNA-seq [27].

### Developmental Biology

177 genes were found to play key roles in the development process, revealed by RNA-Seq used to analyze the transcriptome profiles of four early developmental stages of zebrafish [28]. In channel catfish, to understand the male-heterogametic sex determination mechanism, RNA-seq was used to investigate the whole transcriptome of testis [16]. In this study, 5,450 genes were found preferentially expressed in the testis, and many of these genes were involved in gonadogenesis, spermatogenesis, testicular determination, gametogenesis, gonad differentiation, and possibly sex determination [16].

Overall, transcriptome analysis (RNA-seq) is a powerful tool that can lead to a better understanding of the underlying pathways and mechanisms of many scientific questions related to aquaculture. Although RNA-seq has been used in various research fields in various aquatic animals, including fish, crustaceans, and mollusk, the applications of RNA-seq are currently still limited to a few aquaculture species, and some of these studies are limited to model animals, such as zebrafish. Additionally, the scope of RNA-seq applications must

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extend to other important research fields, such as aquaculture nutrition physiology, which plays important roles in aquaculture. Furthermore, most available studies using RNA-seq technology have reported overall gene and pathway responses for a few biological processes, but the detailed functions or responses of crucial gene or pathway have not been fully studied, therefore, further functional studies should be conducted to validate the results and hypothesis obtained from RNA-seq.

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