



The testis and ovary transcriptomes of the rock bream (*Oplegnathus fasciatus*): A bony fish with a unique neo Y chromosome



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ABSTRACT

The rock bream (*Oplegnathus fasciatus*) is considerably one of the most economically important marine fish in East Asia and has a unique neo-Y chromosome system that is a good model to study the sex determination and differentiation in fish. In the present study, we used Illumina sequencing technology (HiSeq2000) to sequence, assemble and annotate the transcriptome of the testis and ovary tissues of rock bream. A total of 40,004,378 (NCBI SRA database SRX1406649) and 53,108,992 (NCBI SRA database SRX1406648) high quality reads were obtained from testis and ovary RNA sequencing, respectively, and 60,421 contigs (with average length of 1301 bp) were obtained after de novo assembling with Trinity software. Digital gene expression analysis reveals 14,036 contigs that show gender-enriched expression profile with either testis-enriched (237 contigs) or ovary-enriched (581 contigs) with RPKM >100. There are 237 male- and 582 female-abundant expressed genes that show sex dimorphic expression. We hope that the gonad transcriptome and those gender-enriched transcripts of rock bream can provide some insight into the understanding of genome-wide transcriptome profile of teleost gonad tissue and give useful information in fish gonad development.

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Specifications	
Organism/cell line/tissue	<i>Oplegnathus fasciatus</i> /testis and ovary
Sex	Male and female
Sequencer or array type	Illumina Hiseq2000
Data format	Raw and processed
Experimental factors	Transcriptome profiling of testis and ovary at mature stages
Experimental features	Testis and Ovary at mature stages were dissected from the rock bream and the total RNAs were extracted by using TRIZOL reagent. Prepared cDNA libraries were paired-end sequenced by HiSeq2000 system. The obtained data was subjected for de novo transcriptome assembly using Trinity, and coding regions were predicted by BLAST. We performed BLASTx against the NR database with an e-value cut off of 1e-6 for unigene annotation. Gene ontology (GO) analysis was conducted by

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(continued)

Specifications	
Consent	using Blast2GO.
Sample source location	N/A Key Lab of Mariculture and Enhancement of Zhejiang Province, Marine Fisheries Research Institute of Zhejiang Province, Zhoushan, Zhejiang, China

1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/sra/SRX1406648> for male gonad.
<http://www.ncbi.nlm.nih.gov/sra/SRX1406649> for female gonad.

2. Introduction

Teleost fishes are an outstanding model to study the evolution of sex chromosome since they present a broad range of sex chromosome systems, as well as the absence of differentiated sex chromosomes in most species [3]. Although the diversity of sex determination system in fish, it is hypothesized that genes involved in sex determination are probably conserved throughout evolution. To date, master sex-determination

genes have been identified for fish species, including *dmy* in medaka [11,12], *sdv* in rainbow trout [20], *amhr2* in fugu [6] and *amhy* in Patagonian pejerrey [5]. Besides the sex determining genes, some conserved genes shown to play important roles in mammal sex determination and differentiation were analyzed in fish, including *cyp19*, *dax1*, *dmrt*, *foxl2*, *mis*, *sf1*, *sox9* and *wt1* [13]. These genes act together to constitute complicated network whereby sex phenotype is established in mammals. However, studies on the function and connections of the above genes in fish are in their infancy. More or novel sex-related genes are required to be clarified to further explain the complex mechanism of fish sex determination.

Over the past decade, significant progress has been made in genome-wide gene expression profiling by the development and application of large scale sequencing technique, which can easily show more differential expression genes in different traits, such as gender. Transcriptome profiling associated with sex determination and differentiation using RNA-seq were reported in several fish species, including platyfish [21], Nile tilapia [15], catfish [14], and Yellow catfish [9]. These data provided transcriptomic information expressed in gonads at particular condition and time and identified sex differentially expressed genes, which are crucial to providing valuable insight in studying fish sex-determination.

Rock bream (*Oplegnathus fasciatus*), a subtropical and carnivorous species, is an economically important marine fish in East Asia. It has recently been targeted as a promising species for commercial aquaculture and stock enhancement in China. This species possess a differentiated neo-Y chromosome, and is characterized by having a multiple $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system [16,19]. Therefore, rock bream fish is an excellent model with which to understand the molecular mechanism of sex determination and sex chromosome evolution in fishes. To gain a global view of the multiple interrelated molecular changes that relate to the sexual dimorphism in rock bream and provide a database for future studies, we initiated a transcriptome project to obtain deep coverage of cDNAs from adult fish of different gender. Our findings may provide a valuable genomic resource for gene annotation and discovery of genes for sex determination as well as development of molecular makers in rock bream.

3. Experimental design, materials and methods

3.1. RNA extraction

The fully matured testis and ovary were dissected from wild captured rock bream and immediately stored in RNAlater (Qiagen, Hilden, Germany) and then stored at -80°C prior to RNA extraction. At the same time, the gonad maturation of the rock bream was checked by histological section. Sperm can be easily striped from the male individual, and the lobular lumen and sperm duct were filled with spermatozoa under microscope (Fig. 1a), which suggest the male is mature at stage V [10]. For the female individual, postovulatory follicles were observed together with oocytes at yolk stage in histological sections of ovaries, indicating the female at maturity stages V (Fig. 1b). Two male specific markers *Opl286* and *Oplfa16* [17,18] for rock bream sex identification were also used to detect the genetic sex of the fish individuals for RNA-seq (Fig. 1c and d). Total RNAs were extracted by using the TRIZOL Kit (Invitrogen, Carlsbad, CA, USA) following manufacturer's instructions. Total RNA samples were then digested by DNase I to remove potential genomic DNA contamination. Integrity and size distribution were checked with Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, USA).

3.2. RNA isolation, library construction and Illumina sequencing

Initially, about 2.5 μg of starting total RNAs were used to synthesize the cDNA libraries by following the standard protocols of the Illumina TruSeq RNA Sample Preparation Kit (Illumina). The final library had an average fragment size of 180 bp and final yields of ~ 400 ng. After KAPA quantitation and dilution, the library was sequenced on an Illumina HiSeq 2000 with 101 bp paired-end reads. A total of 40,004,378 and 53,108,992 paired-end reads was generated with a read length of 101 bp in the female and male transcriptomes, respectively. The raw transcriptome sequences in the present study was deposited in the NCBI SRA database (SRX1406648 and SRX1406649 for male and female gonads, respectively). Adaptor sequences were trimmed and reads with low quality or length less than 70 were further removed by SolexaQA software. After the removal of ambiguous

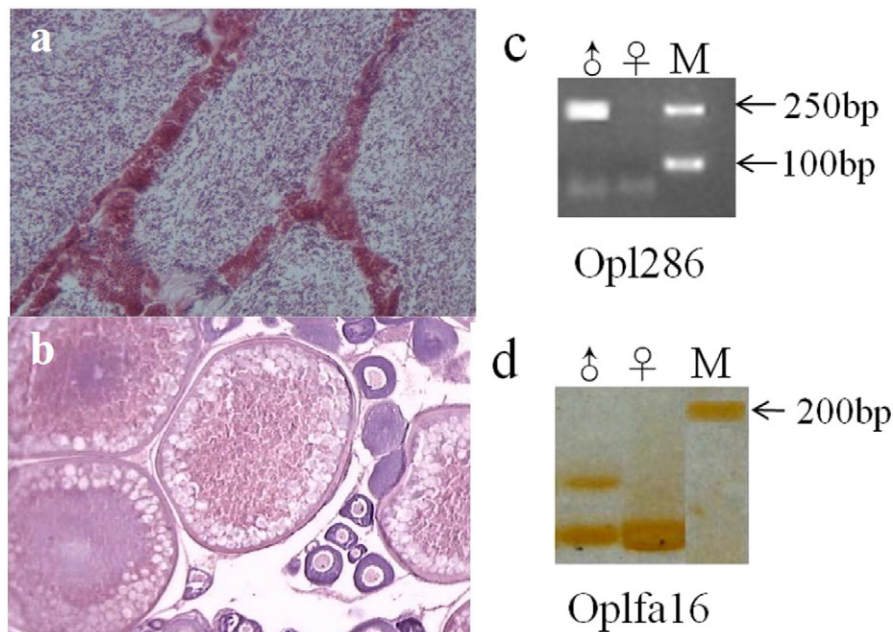


Fig. 1. Gender identification in rock bream by histological section and molecular markers genotyping in rock bream. (a) Paraffin section of mature ovary at stage V, (b) paraffin section of mature testis at stage V. The tissue section was stained with H&E to enhance the contrast. The gender of rock bream is sexing by the male-specific markers of *Opl286* (c) and *Oplfa16* (d).

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