

Figure S1. Eukaryotic base percentage composition along reads. Horizontal axis is base coordinates of reads, which represent the base from the 5' to the 3' end sequentially. Vertical axis is corresponding percentage, each base with different color. A is purple; C is green; G is blue; T is orange; N is yellow. But in existing high-throughput sequencing technologies, reverse transcribed into cDNA was used in 6 bp random primers can cause a few position of nucleotide composition exist certain preferences, which belongs to the normal situation. (A), (B), (C) are wild type strain; (D), (E), (F) are *fliA*-RNAi strain.

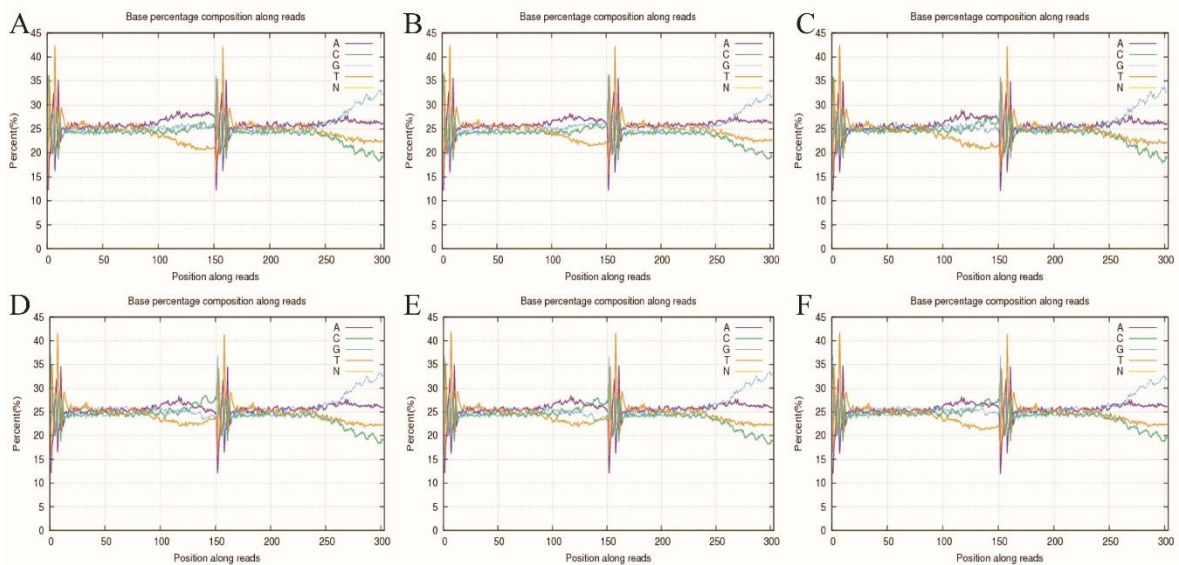


Figure S2. Prokaryotic base percentage composition along reads. Horizontal axis is base coordinates of reads, which represent the base from the 5' to the 3' end sequentially. Vertical axis is corresponding percentage, each base with different color. A is purple; C is green; G is blue; T is orange; N is yellow. But in existing high-throughput sequencing technologies, reverse transcribed into cDNA was used in 6 bp random primers can cause a few position of nucleotide composition exist certain preferences, which belongs to the normal situation. (A), (B), (C) are wild type strain; (D), (E), (F) are *fliA*-RNAi strain.

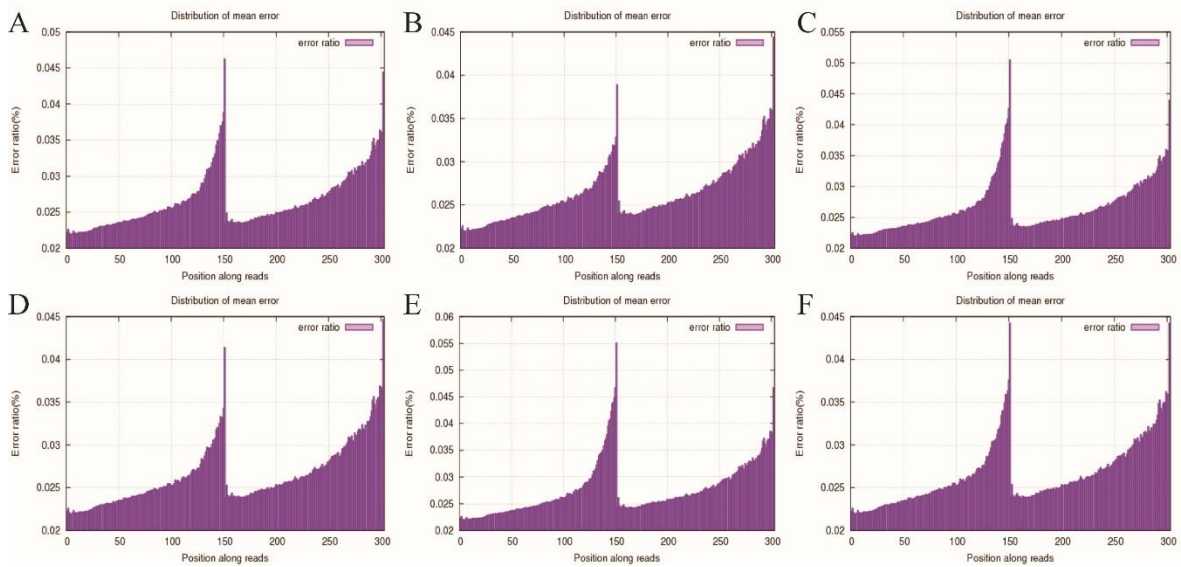


Figure S3. Eukaryotic distribution of mean error. Distribution of mean error. Horizontal axis is base coordinates of reads, which represent the base from the 5' to the 3' endsequentially. Vertical axis is the average base error rate of all reads at the site (%). The purple shadow in the graph corresponds to the average value of the base error rate, which reflects the distribution of base error rate in the sequencing reads, and the acceptable range of the average base error rate is less than 0.1%. (A), (B), (C) are wild type strain; (D), (E), (F) are *fliA*-RNAi strain.

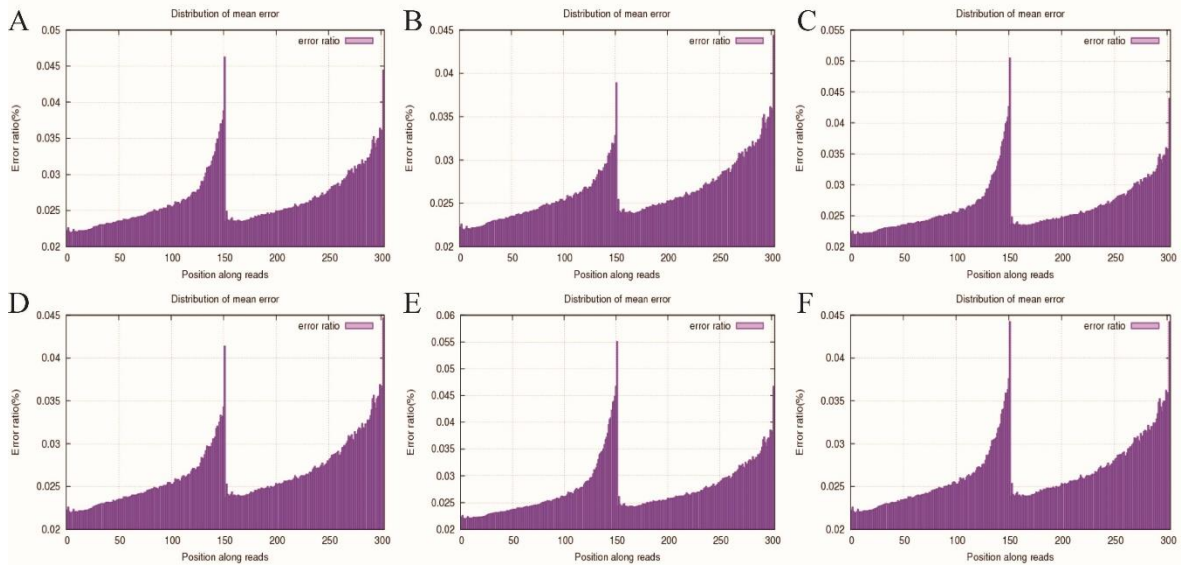


Figure S4. Prokaryotic distribution of mean error. Distribution of mean error. Horizontal axis is base coordinates of reads, which represent the base from the 5' to the 3' endsequentially. Vertical axis is the average base error rate of all reads at the site (%). The purple shadow in the graph corresponds to the average value of the base error rate, which reflects the distribution of base error rate in the sequencing reads, and the acceptable range of the average base error rate is less than 0.1%. (A), (B), (C) are wild type strain; (D), (E), (F) are *fliA*-RNAi strain.

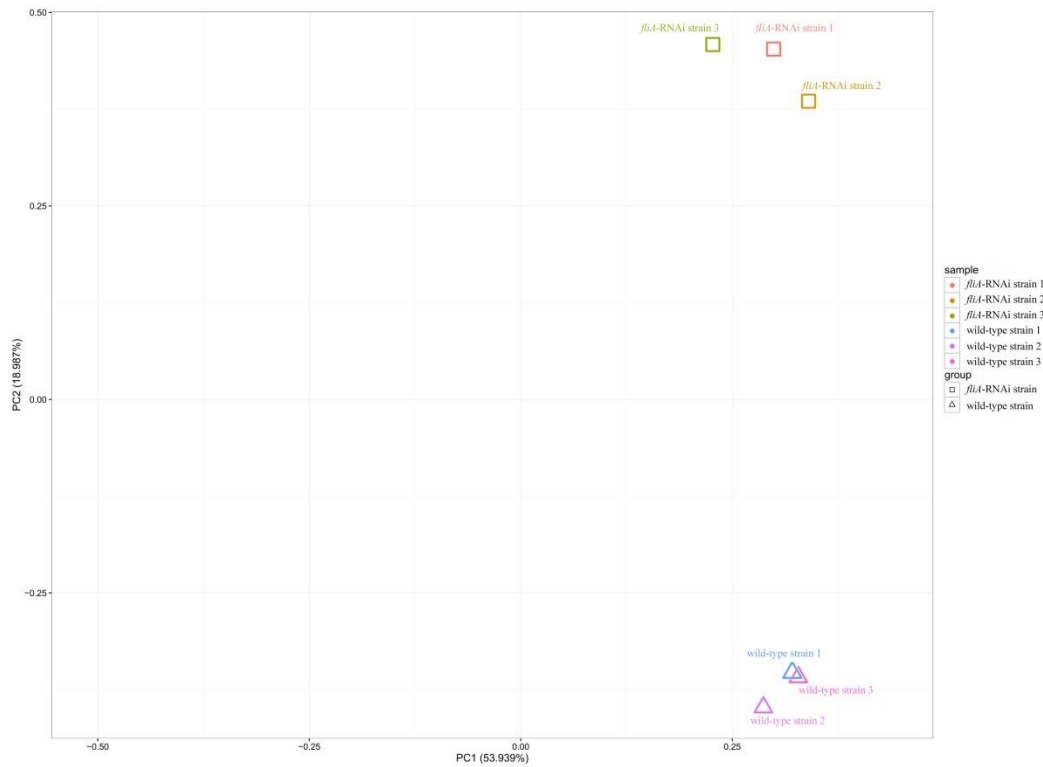


Figure S5. Eukaryotic PCA analysis. Principal component analysis(PCA) can reduce the complexity of data, and dig deep the relation between sample size and variation. The basic principle is that diverse samples have different measurement, PCA is to find out the main factors of observed value differences, considering all the factors are combined and sort according to importance. Usually the tiny factors are ignored, which play a role of simplify the data. For two or three principal components axis graphed, which can see the distance of the relationship between each sample, including visual effect of clusters groups.

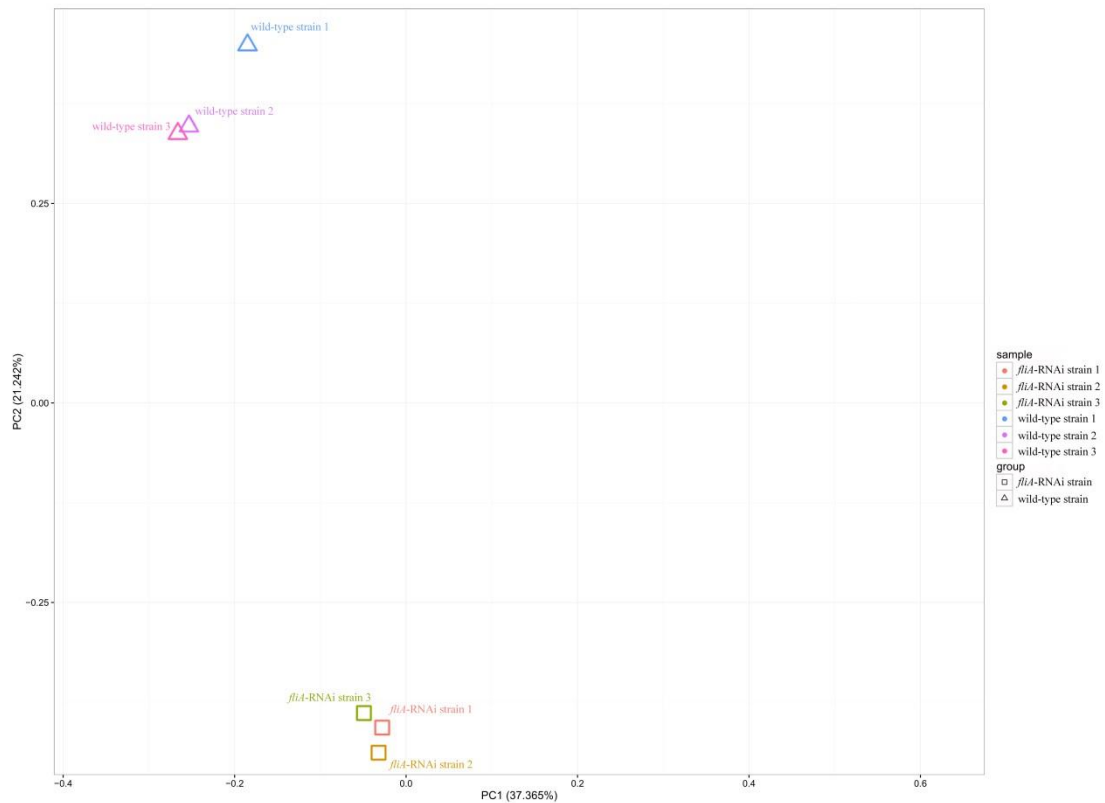


Figure S6. Prokaryotic PCA analysis. Principal component analysis(PCA) can reduce the complexity of data, and dig deep the relation between sample size and variation. The basic principle is that diverse samples have different measurement, PCA is to find out the main factors of observed value differences, considering all the factors are combined and sort according to importance. Usually the tiny factors are ignored, which play a role of simplify the data. For two or three principal components axis graphed, which can see the distance of the relationship between each sample, including visual effect of clusters groups.

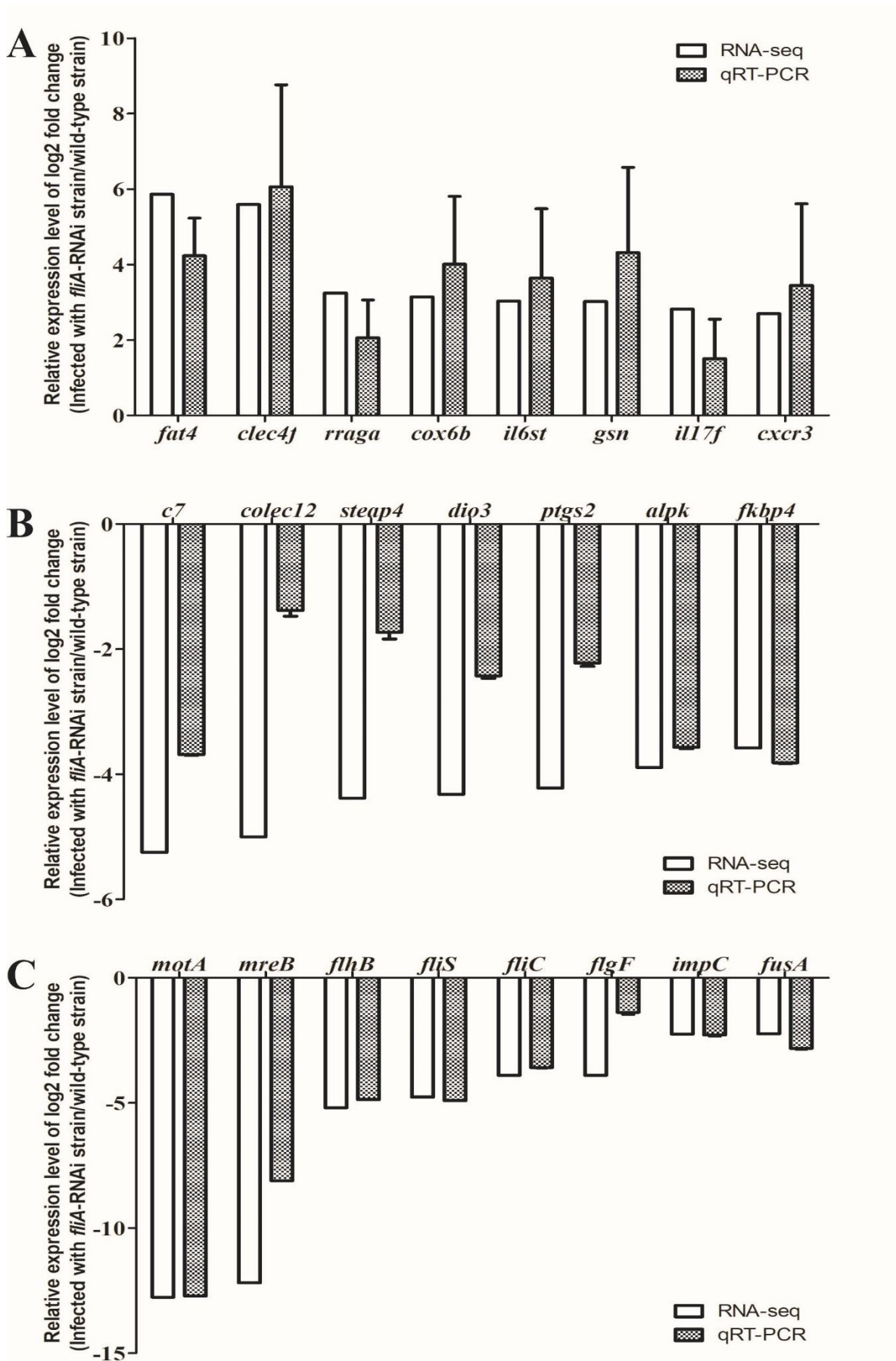


Figure S7. Verification transcriptome data by qRT-PCR. Relative changes in abundance of selected genes. qRT-PCR was performed by triple technical repetitions. White bars: RNA-seq; Grid bars: qRT-

PCR. A: up-regulated genes in eukaryotic transcriptome; B: down-regulated genes in eukaryotic transcriptome; C: down-regulated genes in prokaryotic transcriptome.

Table S1. List of shRNA sequence used to silence *fliA* expression in present study.

Name	Sequence(5'-3' orientation)
shRNA-66	TGCGTTGGTTGATGGACTACCTTTCAAGAGAAGGTAGTCCATCAACCAACGCTTTTTTT GTACAAAAAAGCGTTGGTTGATGGACTACCTTCTCTTGAAAGGTAGTCCATCAACCAACGCATGCA
shRNA-93	TGGTCAAGCGCATTGTCAATCATTCAAGAGATGATTGACAATGCGCTTGACCTTTTTTT GTACAAAAAAGGTCAAGCGCATTGTCAATCATCTCTTGAATGATTGACAATGCGCTTGACCATGCA
shRNA-99	TGCGCATTGTCAATCAGCTATCTTCAAGAGAGATAGCTGATTGACAATGCGCTTTTTTT GTACAAAAAAGCGCATTGTCAATCAGCTATCTCTTGAAGATAGCTGATTGACAATGCGCATGCA
shRNA-445	TGCCATCGAAAGCCTCGATACATTCAAGAGATGTATCGAGGCTTTCGATGGCTTTTTTT GTACAAAAAAGCCATCGAAAGCCTCGATACATCTTGAATGTATCGAGGCTTTCGATGGCATGCA
shRNA-656	TGGGTCTGTCAAGTTGAGTAAGCTTCAAGAGAGCTTACTCAACTGACAGACCCTTTTTTT GTACAAAAAAGGGTCTGTCAAGTTGAGTAAGCTCTTGAAGCTTACTCAACTGACAGACCCATGCA

Table S2. List of qRT-PCR primers used to determine gene expression changes in present study.

Gene name	Forward primer sequence(5'-3' orientation)	Reverse primer sequence sequence(5'-3' orientation)	Source
<i>16S</i>	TCAGTATCAGTCCAGGTGGTCC C	CGTTACCGACAGAATAAGCACC G	(1)
<i>β-actin</i>	GACCTGACAGACTACCTCATG	AGTTGAAGGTGGTCTCGTGGA	(2)
<i>gyrB</i>	TGCTGAAGGACGAGCGTTCG	ATCATCTTGCCGACAACAGC	(3)
<i>fliA</i>	CGAGGGTTTCTTCAGAGCGGTA C	GCACCAGGCCAATTTCTTTAAG AT	This study
<i>C7</i>	GCCGAACCCGTTTACTTG	TCATCGCAGGATATGGAC	This study
<i>il1b</i>	TAAGACGGAAGTTTGGAT	AAGCGTTTCACTTTCTGTAC	This study
<i>colec12</i>	GGAGAATGGAATGGGTG	GGAGCAGGGAACATCGTA	This study
<i>steap4</i>	GAAGGTGCTGGTGGATGT	TCTACTGGCGTCTGAGGG	This study
<i>dio3</i>	GCACCGCTGTTTGGAGGA	CGGCGTTGGATGAGTTGTC	This study
<i>ptgs2</i>	TGATGTTGCGATTCTTCG	TTGCCTTCCAGGTTGTC	This study
<i>alpk</i>	GGAATGGGTAACGTATGT	GAATGGGAACTGAAACTG	This study
<i>fkbp4</i>	CAAGAAGACCTACGCCAATA	CTCCCTTCAAATGTCACCA	This study
<i>fat4</i>	GGAATGGGAGAATGGAAC	ACAAGACAGAACAGGAGC	This study
<i>clec4f</i>	TCTGAGTGACAGCCTTGA	CCTTCCTTCTGCACGATT	This study
<i>rraga</i>	GCGATACGCTCTGTTGAA	ACAGCAAAGACAGCCAC	This study
<i>cox6b</i>	AAAGGGTCTACAAATCAATC	TCTCCCACCACTTCAACA	This study
<i>il16st</i>	GGCCAAGACGACCTCTAACG	GTGCTGTGCTGCCAGTGATT	This study
<i>gsn</i>	GATGATGACAAGGCGGACAC	GTAGCACTCTTCGGGAGACA	This study
<i>il17f</i>	TAGAATCCCGTTTACCTCAG	CTTCCTCCCTCCTTTGTTAT	This study
<i>cxc3</i>	ACGGACACCTTCCTCCTTCA	AACAGCCAATCCAAACACCC	This study
<i>motA</i>	TCTTCCTTTGCTATTGCCTGATG	TGTTTGAACGCTGGCTTGAC	This study
<i>mreB</i>	TCTCGCTGAACGGCGTGGTC	GATGCGCTCGGCGGTGGATT	This study
<i>flhB</i>	TGGTGGTCAATGTGATGGTAGT	GGTGATCGGGTTTAGCTTTT	This study
<i>fliS</i>	AAAGGGAAATCGCTGGAGAA	AACGGTGCAAACAGTAATCGTA T	This study
<i>fliC</i>	GCCTCGTTCCGGTGCCAATCT	CGCTTCCAGGGCGTAGTCG	This study
<i>flgF</i>	CAAGGGCGGCGAAGCCTATA	CCCGTGGCAACACCGAGACA	This study
<i>impC</i>	CGAACTCGTGCCAGAAACCA	CCAGGCGGCTGACCACAAAA	This study
<i>fusA</i>	CCGTCATCAACTCGGTCAAG	CAGGGAAGTCCATACGCTCA	This study

Supplementary references

1. Sun, Y.; Luo, G.; Zhao, L.; Huang, L.; Qin, Y.; Su, Y.; Yan, Q. Integration of RNAi and RNA-seq Reveals the Immune Responses of *Epinephelus coioides* to *sigX* Gene of *Pseudomonas plecoglossicida*. *Front. Immunol.* **2018**, *9*, 1624.
2. Zheng, W.; Liu, G.; Ao, J.; Chen, X. (2006). Expression analysis of immune-relevant genes in the spleen of large yellow croaker (*Pseudosciaena crocea*) stimulated with poly I: C. *Fish Shellfish Immunol.* **2006**, *21*, 414-430.
3. Izumi, S.; Yamamoto, M.; Suzuki, K.; Shimizu, A.; Aranishi, F. Identification and detection of *Pseudomonas plecoglossicida* isolates with PCR primers targeting the *gyrB* region. *J. Fish Dis.* **2007**, *30*, 391–397.