

# STUDIES ON MOLECULAR MARKERS FOR HEMOCYTES IN KURUMA SHRIMP *Marsupenaeus japonicas*

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## ABSTRACT

Major shrimp immune response such as phagocytosis, encapsulation and prophenoloxidase activity take place in the hemocytes. It is said that different kinds of hemocytes play different roles in the immune system therefore their classification are very important for understanding hemocytes function, as well as the entire immune system. Generally, hemocytes have three types of cell, hyaline cell, semigranular cell, and granular cell with reference to morphological features. However, morphological features are ambiguous and results may vary depending on the method used so that an accurate method is needed to establish classification of hemocytes. To resolve this problem, classification up to molecular level using molecular marker should be done. This study aims to develop an accurate classification method through identifying candidate molecular markers of hemocytes. Enable to do this, hemocyte samples were separated into two layers by density gradient centrifugation (upper layer and lower layer). Morphological features of hemocytes in each layer were analyzed by May Giemsa staining and flow cytometry analysis. In the result, hemocytes in upper layer showed larger size of cell and nucleus and less complexity of cell than lower layer. Then, gene expression analysis in each layer was conducted by transcriptome analysis and qPCR. Sixteen genes which showed more than eight times difference in total number of reads with p-value less than  $1 \times 10^{-5}$  were selected from transcriptome analysis. Fourteen genes showed significantly larger number of reads in upper layer and two genes showed larger number of reads in lower layer which was further confirmed by the mRNA levels of these sixteen genes were then analyzed by qPCR, where all genes indicated higher level of mRNA in same layers. These results imply that hemocytes can be separated into two types through morphological features, where sixteen candidate genes were identified to be possible markers, which may further classify hemocytes to different types. These results may provide technical basis for the classification of shrimp hemocytes.

## KEYWORDS

kuruma shrimp, hemocytes, classification, molecular markers, morphological features

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