



1. Background

The Pacific white shrimp (*Litopenaeus vannamei*), as the most widely farmed shrimp species globally, holds significant economic value in the aquaculture industry. However, fundamental biological research, particularly on the epigenetic regulation of embryonic development, remains limited for *L. vannamei*. This study investigated the dynamic changes in histone modifications and gene expression across key embryonic developmental stages in *L. vannamei*, aiming to enhance our understanding of epigenetic regulation in marine invertebrates during embryonic development.

2. The successful application of CUT&Tag technology on *L. vannamei*

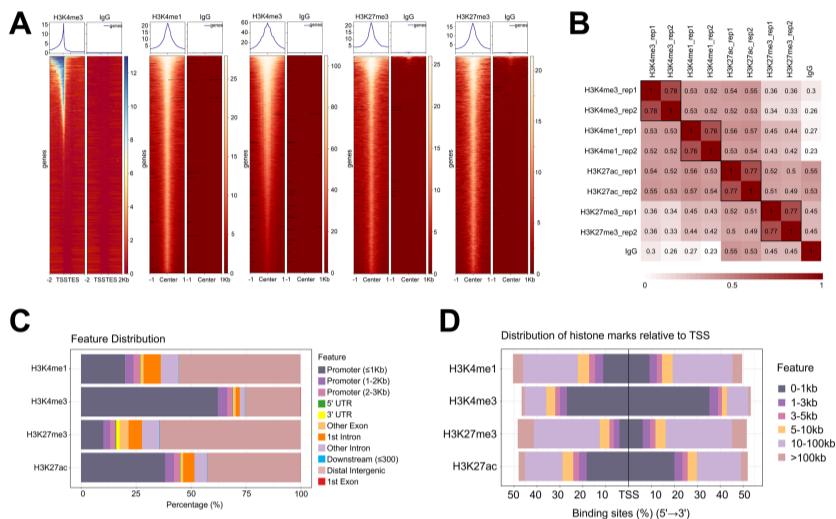


Fig 1. CUT&Tag was successfully established in *L. vannamei* for the first time. (A) The four histone marks investigated showed signal enrichment at specific genomic regions, with H3K4me3 particularly enriched around transcription start sites (TSS), consistent with expectations. (B) Correlation analysis of replicate samples demonstrated good reproducibility. (C-D) The genomic distribution of the four histone modifications aligns with previously established patterns.

3. The dynamic changes in histone modifications and the transcriptome revealed early activation of zygotic genome in *L. vannamei*

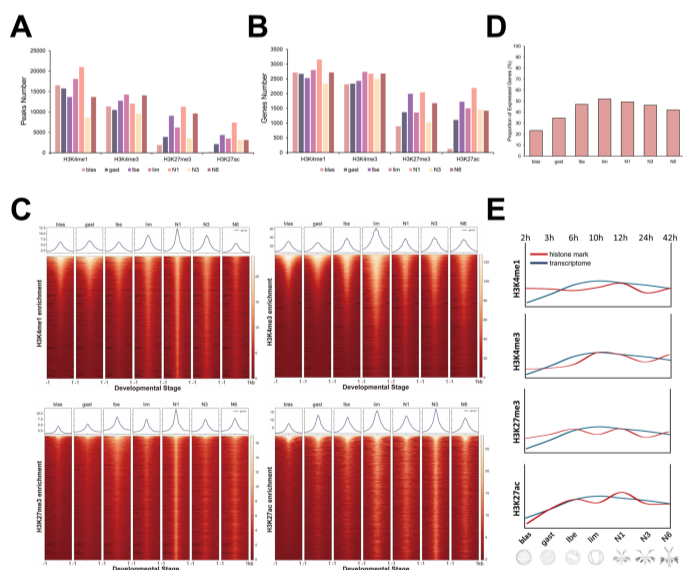


Fig 2. Epigenetic gene regulation characteristics and zygotic genome activation patterns during *L. vannamei* embryonic development. (A-B, C) As early as the blastula stage, numerous gene loci in the *L. vannamei* genome exhibit histone modifications H3K4me1 and H3K4me3, with no significant quantitative changes observed as development progresses. In contrast, histone modifications H3K27me3 and H3K27ac gradually increase only after the gastrula stage. (D) On the transcriptome level, the number of expressed genes begins to increase from the blastula stage, indicating that zygotic genome activation in *L. vannamei* occurs relatively early, potentially related to the presence of H3K4me1 and H3K4me3 modifications. (E) A model of histone modification and transcriptomic changes during *L. vannamei* embryonic development.

7. Conclusion

This study provides a novel method for marine invertebrate research, and offers abundant resources for the study of epigenetic regulatory mechanisms in *L. vannamei* development, as well as for functional genomic research.

4. The co-expression network revealed distinct gene expression patterns associated with developmental progression

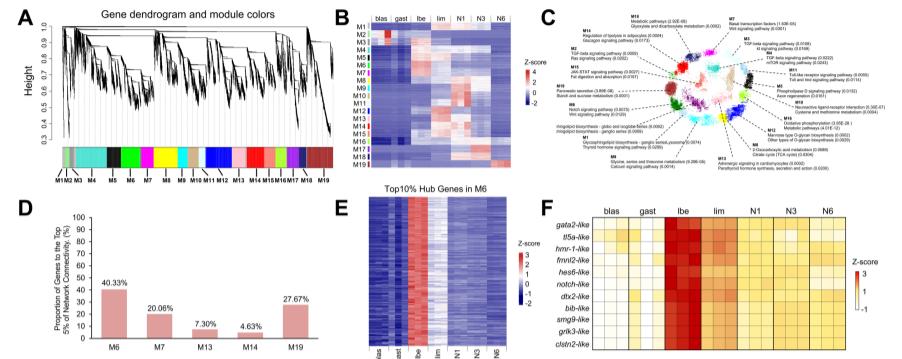


Fig 3. The WGCNA was utilized to construct a gene co-expression network during the embryonic development of *L. vannamei*, identifying 19 gene modules with distinct expression patterns across different developmental stages. KEGG enrichment analysis revealed that the genes within these modules are associated with cellular processes, metabolism, immune system, and nervous system functions. Notably, in the M6 module, key genes such as *gata2*, *tl5a*, and *notch* were highly expressed during the mid to late embryonic stages, indicating their critical roles in cell differentiation, immune system development, and nervous system maturation.

5. The functional enrichment analysis of expressed genes modified by histone modifications

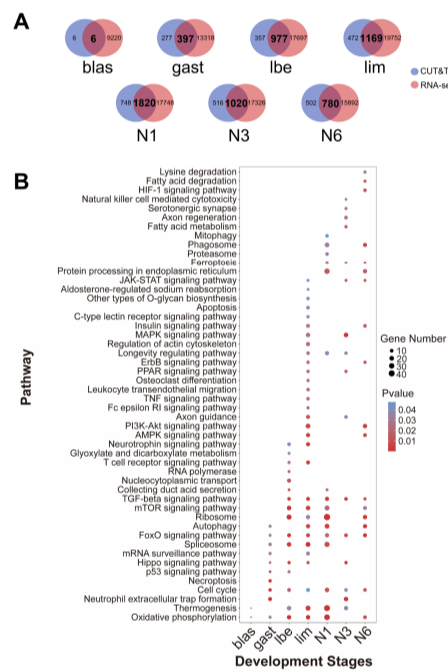


Fig 4. CUT&Tag and transcriptome data were integrated to analyze the dynamics of gene expression regulated by histone modifications during the embryonic development of *L. vannamei*. The results showed that histone modifications at different developmental stages were closely associated with significantly enriched signaling pathways. For example, during the gastrula and limb bud embryo stages, gene expression was primarily related to cell growth and differentiation pathways, while at other stages, it was associated with immune system, metabolism, and signal transduction pathways. By analyzing the histone-modified expressed genes at each developmental stage, the figures revealed the number of these genes and their functional enrichment profiles.

6. Identification of key genes modified by histone modifications

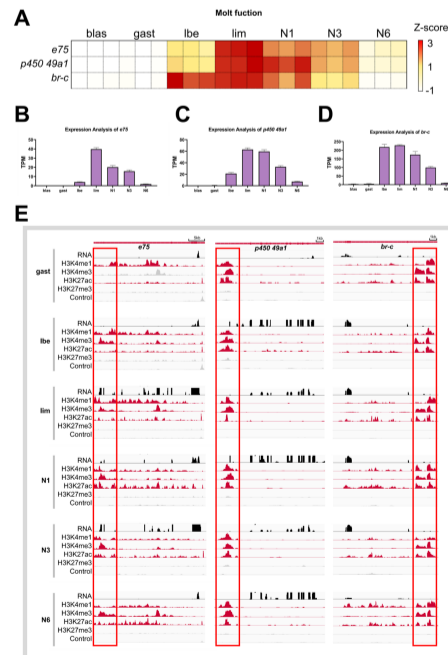


Fig 5. CUT&Tag and transcriptome data were integrated to focus on analyzing key genes closely related to developmental traits during *L. vannamei* embryonic development, such as the molting-related genes shown in the figure. These genes (e.g., *e75*, *p450 49a1*, and *br-c*) were significantly expressed during the later stages of embryogenesis, and their expression levels were highly consistent with the dynamic changes of active enhancer marks (H3K4me1 and H3K27ac). These findings suggest that histone modifications play a crucial role in regulating the transcriptional activity of these molting-related genes.

8. Acknowledgements

This work was supported by the Shandong Provincial Special Funds for Taishan Scholars (tsqn202306104), Project of Sanya Yazhou Bay Science and Technology City (SCKJ-JYRC-2023-62) and National Natural Science Foundation of China (32200667)