文章摘要

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对虾组织样品中RNA的铵盐常温保存法及其效果

Ammonium preservation of shrimp tissue RNA at normal temperature and its effects

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中文摘要:

本研究以凡纳滨对虾为研究对象，取其肌肉组织28℃下保存于不同饱和度和不同pH的铵盐保存液中，保存一定时间后，通过组织总RNA提取、RT-PCR法及荧光定量RT-PCR法鉴定比较选择方便有效的保存方法。结果显示，从含25mmol/L柠檬酸钠和20mmol/L EDTA的饱和醋酸铵保存液和饱和硫酸铵保存液中保存的组织中提取的RNA较完整。通过调节pH进行保存液的优化，提取RNA后的凝胶电泳、RT-PCR及荧光定量RT-PCR确定的基因拷贝数显示，从含25mmol/L柠檬酸钠和20mmol/L EDTA的饱和醋酸铵保存液（pH 6.0）和饱和硫酸铵保存液（pH 5.2）保存的组织中能成功地提取出大量的RNA，且RNA分子完整；通过荧光定量RT-PCR法显示新鲜组织的18S rRNA拷贝数为107Copies/mg组织，保存28d后的组织18S rRNA拷贝数可达到105Copies/mg组织，而空白对照组的18S rRNA拷贝数只有103Copies/mg组织。说明保存液对于抑制核酸降解有较好的效果。这种简便有效的保存液为样品的保存、现场采集标本及后续的分子实验奠定了基础。

英文摘要:

In recent years, more and more nucleic acid based molecular biotechnologies were established and applied in the disease diagnosis, health evaluation, epidemiological surveillance, genetic breeding, environmental analysis, and other aspects of aquaculture. Good quality and high quantity of nucleic acid to be preserved and extracted from the samples are the prerequisites of these technologies. Sample collection and preservation methods are the key elements to ensure the successful application of the technologies. Currently, most studies use fresh material or ultra-low temperature preserved sample for RNA extraction. However, these methods are very difficult to be applied to aquatic animals, especially for field-collected samples. This study aimed to find a convenient and effective preservation solution for field-collection. Muscle tissues were taken from Litopenaeus vannamei and preserved in the ammonium solutions at different saturation and different pH at 28 ℃ for different time periods. Comparison of the RNA extraction showed that preservation solution of saturated ammonium sulfate and saturated ammonium acetate had good effect. The saturated ammonium acetate at pH 6.0 (A3) and the saturated ammonium sulfate at pH 5.2 (S3) with 25mmol/L sodium citrate and 20mmol/L ethylene diamine tetraacetic acid(EDTA) had a significant effect on

tissue preservation, by comparing gene copy number by total RNA extraction, gel electrophoresis, and RT-PCR. We successfully extracted a large amount of genomic RNA, and quantitative results showed that the 18S rRNA copy number reached 105 for per milligram tissue after 4 weeks preservation in A3 and S3, while the negative control 18S rRNA copy number was 103 per milligram tissue. Preservation solutions can effectively inhibit the degradation of nucleic acid and maintain the tissue structure and cell integrity. This simple and effective preservation solution has a great significance for sample preservation, field specimen collection and subsequent molecular experiments.

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