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SSH技术筛选家蝇抗感染差异基因

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SSH screening on Musca domestica differential genes of defense against infection

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摘要 目的 为了解家蝇抗菌分子免疫机制及效应免疫分子的表达情况, 运用ssh、real-time PCR, 构建家蝇差异表达消减文库。方法 以金黄色葡萄球菌(S. aureus)、大肠杆菌(E. coli)和球孢白僵菌(Beauvoir bassinetbacteria), 感染家蝇幼虫构建金黄色葡萄球菌、大肠杆菌和球孢白僵菌感染的差减文库, 成功构建了家蝇抗感染差异表达cDNA文库。结果 文库的阳性克隆进行PCR鉴定主要分布在250~750 bp之间, 随机挑取252个白斑进行测序和同源性分析, 应用反向Northern斑点杂交技术鉴定了35个基因, 其中32个为真阳性, 包括抗菌肽、酶、核糖体蛋白, 以及一些功能不明的蛋白。结论 运用real-time PCR技术分析了三种蛋白基因的表达情况, 结果显示诱导后不同时间点, 不同发育阶段抗菌肽均普遍表达, 但表达水平存在着明显的差异, 诱导后表达明显升高。

关键词: 家蝇 抗感染免疫 抑制性消减杂交 实时定量荧光PCR

Abstract: In order to identify immune-related genes in housefly (Musca domestica) larvae, suppression subtractive hybridization (SSH) was performed to generate subtracted cDNA libraries after bacteria-

challenge by Staphylococcus aureus (S. aureus), Escherichia coli (E. coli) and Beauvoir bassinet bacteria. Differentially screening was performed using the reverse northern dot blot method for further verification. Of 252 positive clones were obtained; identification of the inserted cDNA fragments in subtractive library was done by using PCR. The results showed that there were inserted fragments of 250-750 bp, which would provide useful baseline for the screening and cloning of specific anti-infection genes of immunity in Musca domestica. Quantitative real-time PCR was used to study the gene expression of AMPs as examples of immune-relevant molecules. It's suggested that the antibacterial peptide genes expression of Musca domestica is constitutive, and they are expressed at different development stages of Musca domestica, but the expressing levels are evidently different.
Keywords： Musca domestica anti-infection immunity suppression subtractive hybridization (SSH) real-time PCR
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