南方水产科学 » 2015, Vol. 11 » Issue (6): 34-40 DOI: 10.3969/j.issn.2095-0780.2015.06.005

罗非鱼无乳链球菌强毒株基因组表达文库的构建及鉴定

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Construction and identification of genomic expression library of *Streptococcus agalactiae* virulent strain Hod-1 isolated from *Oreochromis niloticus*

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摘要 图/表 参考文献(0) 相关文章 (15)

全文: PDF (1304 KB) HTML (0)
输出: BibTeX | EndNote (RIS)

摘要

为了筛选罗非鱼（*Oreochromis niloticus*）无乳链球菌（*Streptococcus agalactiae*）在鱼体内特异诱导表达基因，该研究构建了罗非鱼无乳链球菌强毒株Hod-1的基因组表达文库。采用*Sau*3A Ⅰ对无乳链球菌菌株Hod-1基因组DNA进行不完全酶切，回收大小为0.5~2.0 kb的DNA片段，并与经*Bam*H Ⅰ单酶切且去磷酸化的pET-28 a/b/c表达载体连接，转化表达宿主菌BL21（DE3）。结果显示，所构建的无乳链球菌菌株Hod-1基因组表达文库含1.024×10^5个克隆，远大于覆盖无乳链球菌全基因组所需的最小库容量（45 579个）。PCR检测结果显示重组子中外源片段的插入率为94%，插入片段大小为0.5~2.0 kb，且分布均匀，测序结果显示插入序列与GenBank中无乳链球菌基因组序列同源性高达99%以上。结果表明该研究成功构建了罗非鱼无乳链球菌的基因组表达文库，该文库可用于无乳链球菌在罗非鱼体内诱导表达基因的筛选。

关键词 ： 尼罗罗非鱼，无乳链球菌，基因组表达文库

Abstract :

To screen the specific genes of *Streptococcus agalactiae* induced in *Oreochromis niloticus*, the genomic expression library of the virulent strain Hod-1 was constructed. The *Sau*3A Ⅰ was used to digest genomic DNA of *S.agalactiae* strain Hod-1 incompletely. The DNA fragments ranging from 0.5 kb to 2 kb were recycled and ligated into pET-28 a/b/c expression vectors which had been digested by *Bam*H Ⅰ and dephosphorylated by SAP before using. The recombinant vectors were transformed into BL21 (DE3). The results show that the genomic expression library of *S.agalactiae* contained 1.024×10^5 clones, considerably more than the minimum number of clones (45 579 clones) covering the whole genomic DNA of *S.agalactiae*. Results of PCR detection show that the rate of fragment insertion was 94% and different sizes of inserted fragments ranged from 0.5 kb to 2.0 kb. The results of sequencing show that the homology between inserted sequences and the genomic DNA sequnce of *S.agalactiae* was over 99%. In conclusion, the genomic expression library of *S.agalactiae* strain Hod-1 was constructed successfully, which can be used for screening genes of *S.agalactiae* induced expression in *O.niloticus*.

Key words : *Oreochromis niloticus* *Streptococcus agalactiae* genomic expression library

收稿日期: 2015-01-19 **修回日期:** 2015-03-17 **出版日期:** 2015-12-05

PACS: S 917.1

基金资助:

国家自然科学基金项目（31402295）；现代农业产业技术体系建设专项资金（CARS-49）；农业部淡水渔业和种质资源利用重点实验室开放课题（KF201309）；广州市科技计划项目（2013J4100078，201300000064）

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引用本文:

刘志刚 可小丽 卢迈新 方伟 王淼 朱华平 高风英 曹建萌. 罗非鱼无乳链球菌强毒株基因组表达文库的构建及鉴定[J]. 南方水产科学, 2015, 11(6): 34-40. LIU Zhigang , KE Xiaoli , LU Maixin , FANG Wei , WANG Miao , ZHU Huaping , GAO Fengying, CAO Jianmeng.. Construction and identification of genomic expression library of *Streptococcus agalactiae* virulent strain Hod-1 isolated from *Oreochromis niloticus*. South China Fisheries Science, 2015, 11(6): 34-40.

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