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丙肝病毒融合抗原基因NS3-C定点整合入衣藻叶绿体基因组的研究 NS3-C Chimeric Antigen Gene of Hepatitis C Virus was Introduced Site-specifically into Chloroplast Genome of Chlamydomonas reinhardtii

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收稿日期 修回日期 网络版发布日期 接受日期

摘要 用PCR方法从丙型肝炎病毒(HCV) cDNA文库中克隆了两段DNA片段,即HC 基因组非结构NS3区抗原基因(约0.7 kb)和核心抗原C区抗原基因(约0.6 kb)的cDNA片段。在两段cDNA间加入连接肽Ser-Pro-Gly-Ser的密码子序列,构建成融合抗原基因NS3-C。将该融合基因与衣藻叶绿体基因atpA的启动子和rbcL基因的3'末端连接,得到丙肝病毒融合抗原基因NS3-C表达盒,再将该表达盒与选择标记基因aadA表达盒和衣藻叶绿体基因组同源片段连接,构建成衣藻叶绿体转化载体pSS6。基因枪法转化衣藻叶绿体,经壮观霉素筛选获得转化再生的单藻落,对转基因衣藻的PCR和Southern杂交分析表明,融合抗原基因NS3-C已整合到衣藻叶绿体基因组中。

Abstract: Two DNA fragments encoding the nucleocapsid (C) region protein and the non-structural region 3 (NS3) protein of hepatitis C virus(HCV) were amplified from cDNA library by using PCR method. The 5' terminal of C cDNA fragment was linked up with the 3' terminal of NS3 cDNA fragment by a oligonucleotide linker Ser-Pro-Gly-Ser to form a chimeric gene NS3-C, which was placed under the control of the chloroplast atpA promoter and rbcL 3' region of Chlamydomonas reinhardtii to construct the chimeric gene NS3-C cassette. Then the NS3-C cassette was linked with selectable gene aadA cassette and the chloroplast homologous fragments of Chlamydomonas reinhardtii to generate transformation vector pSS6. Chloroplasts of Chlamydomonas reinhardtii were transformed by particle bombardment. Plastid transformants were selected by their resistance to 100 mg/L of spectinomycin. PCR and Southern hybridization analysis showed that the chimeric gene NS3-C had been integrated into chloroplast genome of Chlamydomonas reinhardtii.

关键词 丙型肝炎病毒 基因融合 叶绿体转化 aadA基因 衣藻 Key words Hepatitis C virus Gene fusion Chloroplast transformation aadA gene Chlamydomonas reinhardtii

分类号

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