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论著

融合基因IFN-α1b/CSPⅡ原核表达载体的构建及在大肠埃希菌中的表达

陈慧红[1],余新炳[2],高兴政[1]

1 北京大学医学部寄生虫学教研室，北京 100083

2 中山大学中山医学院寄生虫学教研室，广州 510089

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摘要

目的 构建融合基因IFN α1b/CSPⅡ的原核表达载体并予以表达。 方法 采用聚合酶链反应(PCR)从人基因组DNA中扩增出IFN α1b基因 ,克隆入原核表达载体 pGEX 4T 1,构建原核表达载体 pGEX 4T 1/IFN α1b。利用PCR法从恶性疟原虫基因组DNA中扩增出环子孢子蛋白Ⅱ区 (CSPⅡ)基因 ,克隆入原核表达载体 pGEX 4T 1,构建原核表达载体pGEX 4T 1/CSPⅡ 。用限制性内切酶BamHⅠ和EcoRⅠ将IFN α1b从原核重组质粒 pGEX 4T 1/IFN α1b中切下 ,克隆入经相同酶切的原核重组质粒pGEX 4T 1/CSPⅡ 中 ,构建融合基因的原核表达载体 pGEX 4T 1/IFN α1b/CSPⅡ。融合基因IFN α1b/CSPⅡ经异丙基 β D硫代半乳糖苷 (IPTG)诱导 ,在大肠埃希菌中进行初步表达。结果 构建的原核表达载体 pGEX 4T 1/IFN α1b、pGEX 4T 1/CSPⅡ和 pGEX 4T 1/IFN α1b/CSPⅡ经PCR和酶切鉴定与预期结果一致。证实融合基因IFN α1b/CSPⅡ拼接成功并正确地克隆入原核表达载体。在大肠埃希菌中表达出融合蛋白IFN α1b/CSPⅡ ,该融合蛋白经十二烷基磺酸钠 聚丙烯酰胺凝胶电泳 (SDS PAGE)分析与理论预测值相符。经蛋白质印迹法 (Westernblotting)鉴定具有免疫原性。 结论 构建了融合基因IFN α1b/CSPⅡ的原核表达载体 ,并在大肠埃希菌中表达了。

关键词 融合基因IFN-α1b/CSPⅡ 环子孢子蛋白Ⅱ 基因重组 序列分析 基因表达

分类号

Construction of Prokaryotic Expression Vector of the Fusion Gene IFN-α1b/CSPⅡ and Expression in *E.coli*

CHEN Hui-hong[1],YU Xin-bing[2],GAO Xing-zheng[1]

Department of Parasitology,Health and Science Center of Peking University,Beijing 100083,China

Abstract

Objective To Construct the prokaryotic expression vector of the fusion gene IFN-α1b/CSPⅡ. MethodsIFN-α1b was amplified from the human genomic DNA by PCR and cloned into prokaryotic expression vector pGEX-4T-1. The recombinant plasmid pGEX-4T-1/IFN-α1b was constructed. Circumsporozoite proteinⅡ(CSPⅡ) was amplified from the *Plasmodium falciparum* genomic DNA by PCR and was cloned into the prokaryotic expression vector pGEX-4T-1. The recombinant plasmid pGEX-4T-1/CSPⅡ was constructed. IFN-α1b was cut from the recombinant plasmid pGEX-4T-1/IFN-α1b digested with BamHⅠ and EcoRⅠ and ligated with the recombinant plasmid pGEX-4T-1/CSPⅡ also digested with *Bam*HⅠ and *EcoR*Ⅰ. The recombinant prokaryotic plasmid pGEX-4T-1/IFN-α1b/CSPⅡ was constructed. The fusion gene IFN-α1b/CSPⅡ was expressed in E.coli by IPTG. Results The prokaryotic expression vector pGEX-4T-1/IFN-α1b, pGEX-4T-1/CSPⅡ and pGEX-4T-1/IFN-α1b/CSPⅡ were identified by PCR, enzyme digestion and gene sequencing. The expressed fusion protein/IFN-α1b/CSPⅡ in E.coli was identified by SDS-PAGE and Western blot. Conclusion The prokaryotic expression vector of the fusion gene IFN-α1b/CSPⅡ was successfully constructed, which was then expressed in *E.coli* .

Key words Fusion gene IFN-α1b/CSPⅡ Circumsporozoite proteinⅡ(CSPⅡ) Recombinant DNA Sequence analysis Gene expression

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通讯作者

作者个人主页 陈慧红[1];余新炳[2];高兴政[1]