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细粒棘球绦虫重组BCG-EgG1Y162菌株的构建和表达

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Construction and Expression of the Echinococcus granulosus Recombinant BCG-EgG1Y162

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摘要 目的 构建和表达细粒棘球绦虫重组卡介苗（BCG）菌株rBCG-EgG1Y162。 方法 通过基因工程技术将细粒棘球绦虫抗原EgG1Y162的编码基因与大肠埃希菌（E. coli）-分枝杆菌穿梭表达质粒载体pMV361重组，并转化E. coli后进行扩增。重组质粒pMV-EgG1Y162经PCR和双酶切鉴定后，进行测序。将鉴定正确的rpMV-EgG1Y162通过电穿孔技术转化至感受态BCG菌株中，构建rBCG-EgG1Y162。经PCR和双酶切鉴定正确后，扩增培养2周，并于45 ℃放置30 min，诱导目的蛋白表达，十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）分析蛋白表达情况，并以兔抗原核表达重组蛋白EgG1Y162血清为一抗进行蛋白质印迹（Western blotting）分析。 结果 重组质粒rpMV-EgG1Y162经PCR扩增和双酶切后，均获得约360 bp的EgG1Y162目的基因片段，与预期片段长度一致，测序结果表明插入序列正确。将其通过电穿孔转化BCG菌株后，rBCG-EgG1Y162生长良好，经酶切和PCR鉴定正确。SDS-PAGE和Western blotting结果显示，目的表达产物的相对分子质量（Mr）约为71 000。 结论 构建和表达了细粒棘球绦虫rBCG-EgG1Y162菌株。

关键词： 细粒棘球绦虫 EgG1Y162 重组卡介苗

Abstract: Objective To construct and express Echinococcus granulosus recombinant bacille Calmette-Guerin （BCG） strain rBCG-EgG1Y162. Methods The encoding gene of the antigen EgG1Y162 of E. granulosus was recombined with E. coli-Mycobacterium shuttle expression plasmid vector pMV361 by genetic engineering technique, and transformed into E. coli for amplification. The recombinant plasmid rpMV-EgG1Y162 was identified by PCR, double digestion with restriction enzymes, and sequence analysis. The confirmed rpMV-EgG1Y162 was transformed into BCG strain via electroporation technique to construct the recombinant rBCG-EgG1Y162. After identification by PCR and double digestion with restriction enzymes, the recombinant strain was cultured for about 2 weeks. In order to induce the expression of target protein, the rBCG was placed in 45 ℃ for 30 min. SDS-PAGE and Western blotting were used to analyze the expressive protein. Results The product of recombinant plasmid rpMV-EgG1Y162 was approximately 360 bp by PCR amplification and double digestion with restriction enzymes, consistent with the expected fragment length. Sequencing results showed that the inserted sequence was correct. The rBCG-EgG1Y162 grew well and the identification of PCR and enzyme digestion revealed accuracy. The results of SDS-PAGE and Western blotting showed that the relative molecular weight（Mr） of the protein was about 71 000. Conclusion The E. granulosus rBCG-EgG1Y162 strain is constructed and expressed.

Keywords: Echinococcus granulosus；EgG1Y162；Recombinant bacille Calmette-Guerin

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