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研究论文

口蹄疫病毒3ABC基因截短体在毕赤酵母中的表达及鉴定

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摘要 将长为525 bp的口蹄疫病毒3ABC基因截短体克隆到毕赤酵母表达载体pPIC9K中, 构建了重组表达质粒pPIC9K-3ABCt. 用*Bgl*Ⅱ线性化后, 电转化毕赤酵母菌GS115, 经表型筛选, PCR鉴定, 获得阳性重组菌(GS115/pPIC9K-3ABCt). 然后进行诱导表达, 通过SDS-PAGE和Western blot鉴定表达产物. 结果表明, 重组菌株成功分泌表达了分子量为40000, 具有免疫反应活性, 且呈二聚体形式的目的蛋白. 在96 h时表达量达到最高峰, 占分泌总蛋白的18%, 达到23.4 mg/L. 为进一步研制口蹄疫免疫和感染动物鉴别诊断试剂奠定了基础.

关键词 口蹄疫病毒(FMDV) 3ABC基因 毕赤酵母 分泌表达 诊断抗原

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Expression and Identification of FMDV 3ABC Truncated Gene in *Pichia Pastoris*

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Abstract Foot-and-mouth disease virus(FMDV) is an important pathogen worldwide; consequently, an important goal is the developments of diagnostic methods. To acquire an optimal diagnostic antigen allows one to distinguish vaccinated anionals from infected animals, a recombinant expression plasmid pPIC9K-3ABCt was constructed by inserting of FMDV 3ABC truncated gene(525 bp) into yeast expression vector pPIC 9K. Se-condly, plasmid pPIC9K-3ABCt was linearized by BglII, and transformed into GS115 cells by electroporation. Positive clones were selected with MD/MM plates and confirmed by PCR and RT-PCR. Finally, expression product of 3ABCt was analyzed by SDS-PAGE and Western blot. The results show that the induced recombinant *Pichia pastoris* GS115/pPIC9K-3ABCt could secret 3ABC protein mainly in dimer form into culture supernatant, which had good immunoreactivity and antigen specificity and its molecular weight output was about 40000. Expression of 3ABCt protein reached peek at 96 h after induction, maximum expression was accumulated up to 18% of the total supernatant protein, and production was about 23.4 mg/L.

Key words Foot-and-mouth disease virus(FMDV) 3ABC Truncated gene *Pichia pastoris* Secretory expression Diagnostic antigen

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