



白纹伊蚊唾液三磷酸腺苷二磷酸酶在毕赤酵母中的分泌表达

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Secretory Expression of Salivary ATP Diphosphohydrolase(Apyrase)from Aedes albopictus in Pichia pastoris

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摘要

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摘要 采用RT-PCR技术克隆编码白纹伊蚊(*Aedes albopictus*) 唾液三磷酸腺苷二磷酸酶(apyrase)的成熟肽cDNA序列, 并克隆至毕赤酵母(*Pichia pastoris*)组成型分泌表达载体pGAPZα-A的α-factor信号肽序列的下游, 构建pGAPZα-A-apyrase重组分泌表达载体。表达载体经Bln I线性化处理后电击转化毕赤酵母GS115感受态细胞, 转化子经Zeocin抗性筛选和菌落PCR, 成功构建了pGAPZα-A-apyrase/GS115工程菌。十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE)结果显示, pGAPZα-A-apyrase/GS115的工程菌分泌表达了相对分子质量(Mr)约为60 000的重组apyrase蛋白。

关键词: 白纹伊蚊 ATP-二磷酸酶 毕赤酵母 表达

Abstract: The gene-coding mature apyrase protein from *Aedes albopictus* was amplified by RT-PCR and cloned in frame with the α-factor secretion signal peptide into *Pichia pastoris* secreting expression vector pGAPZα-A resulting in the pGAPZα-A-apyrase. After being linearized by Bln I restriction enzyme, the recombinant pGAPZα-A-apyrase was transformed into *Pichia pastoris* GS115 by electroporation. Recombinant strains pGAPZα-A-apyrase/GS115 were screened on YPDS plates containing Zeocin and identified by PCR. The recombinant protein of apyrase (Mr 60 000) has been expressed in the supernatant of *Pichia pastoris*.

Keywords: *Aedes albopictus* Apyrase *Pichia pastoris* Secreted expression

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