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基础研究

大肠杆菌β-半乳糖苷酶ED和EA的克隆、表达及活性测定

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摘要:

目的：制备大肠杆菌β-半乳糖苷酶（β- galactosidae）酶供体(ED)和酶受体(EA)片段，获得具有全酶活性的β-半乳糖苷酶。方法：以pSV-β- galactosidase control vector为模板设计引物，用PCR方法获得ED和EA片段DNA序列，插入克隆载体pGEM-T-easy中，获得重组质粒，经酶切、PCR及测序鉴定正确后，将酶切目的片段分别插入原核表达载体pET20b+，并转化大肠杆菌BL21，通过IPTG诱导表达出ED、EA融合蛋白，以亲和层析纯化蛋白，通过ED与EA蛋白形成β-半乳糖苷酶、全酶活性试验来判断ED、EA的功能。 结果：获 得与pSV-β-galactosidase control vector一致的ED、EA碱基序列，构建了重组表达质粒pET20b-ED及pET20b-EA，并分别转化入大肠杆菌DE3，以IPTG诱导行SDS-PAGE电泳，在相对分子质量为14 000及 116 000 处分别可见ED蛋白和EA蛋白条带，应用镍凝胶亲和层析纯化获得目的蛋白。结论：成功地制备了ED、EA蛋白，形成全酶活性的β-半乳糖苷酶。

关键词： β-半乳糖苷酶；酶受体；酶供体；免疫测定

Cloning| expression and activity assay of ED/EA of β- galactosidase in E.coli

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Abstract:

Abstract:Objective
To obtain recombinant enzyme donor (ED) and enzyme acceptor (EA) fragments of β-galactosidase in E.coli and establish new cloned enzyme donor immunoassays (CEDIA) for clinical use. Methods The encoding sequences of ED and EA fragments were amplified with pSV-β- galactosidase control vector as template and inserted into pGEM-Teasy vector,the target gene was chosen by double digestion,PCR and sequencing,then ED and EA fragments were inserted into the expression vector pET20b+. The competent cells of host strain of BL21 were transformed by the recombinant plasmid.The expression of the target protein was induced with IPTG and purified by Ni 2+ -NTA agarose column.The β-galactosidae with activity is formed.
Results The cloned fragments of ED and EA were 100% consistent with that of pSV-β-galactosidase control vector.The expression vector pET20b-ED and pET20b-EA were constructed and expressed.The target protein was purified by Ni 2+ -NTA agarose column.The expressed fusion-protein ED fragment was 14 000 and EA fragment was 116 000 in SDS-PAGE as expected.Conclusion ED and EA proteins are prepared successfully and β-galactosidase with activity is formed.

Keywords: β-galactosidase；enzyme acceptor；enzyme donor；immunoassay

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