

pET28a-TAT-LacZ重组子的构建

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摘要 为了构建高表达pET28a-TAT-LacZ重组子, 观察表达的融合蛋白TAT-β-Gal能否穿过生物膜, 使用人工合成编码TAT蛋白转导区的DNA片段, 插入载体pET28a组氨酸编码区后再连接lacZ基因, 组成pET28a-TAT-LacZ重组表达子, 转化大肠杆菌, 利用组氨酸亲和层析柱纯化TAT-β-Gal融合蛋白, 将融合蛋白加入培养的平滑肌细胞。得到高度纯化的、有活性的TAT-β-Gal融合蛋白, TAT-β-Gal在短时间内进入体外培养平滑肌细胞, 成功地构建了高表达pET28a-TAT-LacZ重组子, 并在体外培养的细胞中证实TAT-β-Gal融合蛋白穿透生物膜的能力, 为肽类、生物大分子药物进入组织细胞内发挥治疗作用提供了理论基础。

关键词 [pET28a质粒](#) [TAT蛋白转导区](#) [TAT-β-Gal融合蛋白](#) [平滑肌细胞](#)

分类号

Constructing the recombinant of pET28a-TAT-LacZ

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Abstract

A synthesized double-stranded oligomeric nucleotide encoding 11-amino acid TAT protein transduction domain³ was inserted into pET28a vector after 6 histidine coding sequence, LacZ gene from pcDNA4/Myc-His/LacZ was digested by EcoR I and HindIII, then cloned into pET28a-TAT. The highly expressed TAT-β-galactosidase was purified by affinity chromatography. TAT-β-Gal fusion protein can cross vascular smooth muscle cells in vitro. This result open new possibility for direct delivery of protein into tissues for therapy.

Key words [pET28a phage](#) [TAT protein transduction domain³](#) [TAT-β-Gal fusion protein](#) [Vascular smooth muscle cells](#)

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