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端粒酶反转录功能区基因的表达和纯化

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摘要　通过ＰＣＲ技术扩增出hTRT的目的片段，克隆至表达载体ｐＥＴ28-b中并转化至ＢＬ２１（ＤＥ３），经ＩＰＴＧ诱导后在52 ｋＤａ处发现有外源基因的表达，密度扫描显示表达蛋白含量为２０％。目标蛋白以包含体形式存在，含８mol/L尿素和１０mmol/L ＤＴＴ的裂解缓冲液溶解的包含体采用金属螯合层析有效分离出目标蛋白。免疫印迹实验表明：诱导出的融合蛋白是端粒酶反转录功能区基因所编码的蛋白质。

Expression and Purification of Telomerase Resever Transcriptase Motifs in E.coli

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Abstract:our project is designed to clone a 1.3kb gene fragment of telomerase catalytic subunit gene which contains seven reverse transcriptase motifs and specific region with conserved sequence termed “T motif”.The gene fragment was amplified by PCR and was inserted into expression vector pET28-b.The recombinant plasmid was induced by IPTG for 4h and a 52KD recombinant protein was produced.Amount of hTRT recombinant protein expression was 20% of total bacterial protein in the form of inclusion.Inclusion was dissolved in 8mol/L urea and 10mmol/L DTT and carried out affinity purification under denaturing condition.The purified hTRT recombinant protein was conformed by Western-blot successfully.

Key words：hTRT resever transcriptase motif; pET vector; affinity purification

关键词　端粒酶　反转录功能区基因　pET 载体　亲和层析

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