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研究报告

pBR322-Red介导的E. Coli染色体基因敲入、位点及表达研究

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摘要 应用pBR322-Red介导的重组工程系统，Kan/sacB选择反选择系统，双链线性DNA重组技术和重叠引物介导的DNA重组技术，将长度为1 653 bp的luc报告基因分别敲入到E.coli W3110染色体lacZ, lacY和lacA基因的位置，建立了一系列具有新遗传表型的菌株：CWL2、CWL4和CWL6。荧光素酶分析表明，外源报告基因luc能在这3个结构基因处有效的组成型表达。为了进一步确定外源基因的表达情况，用霍乱毒素B亚单位基因ctxb替换了lacZ基因，构建了新菌株CWD1。证明了以单拷贝形式存在在大肠杆菌染色体CWD1上的ctxb基因能有效的表达CTB蛋白并能将其分泌至细胞外培养液中。结果初步确定了大肠杆菌染色体上的lac操纵子结构基因位点适合外源基因的敲入和表达。

关键词 pBR322-Red 重组工程；基因敲入； 霍乱毒素B亚单位

分类号 Q87

pBR322-Red Mediated Gene Knockin，Sites And Expression in E．coli Chromosome

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Abstract

Genes lacZ,lacY and lacA in the lac opron of E. coli chromosome were respectively substituted with gene luc by using plasmid pBR322-Red, selection-counterselection system kan/sacB and various strategies of Red homologous recombination including Red mediated linearized double-stranded DNA homologous recombination and Red mediated recombineering with overlapping single stranded DNA oligonucleotides.Then, a series of new strains, CWL2,CWL4 and CWL6, were constructed and we found that they can express protein Luc efficiently. To further study the expression of exogenous genes at the site of lacZ, we have constructed a strain named CWD1 by knockin the cholera toxin B subunit(ctxb) gene at the lacZ site, then we found that CWD1 can express protein CTB efficiently and CTB was secreted out of the cell. So we assured that the sites of structure genes in the lac operon of Escherichia coli chromosome were suitable for expressing foreign genes .

Key words pBR322-Red recombineering gene knockin Cholera toxin B subunit

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