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带有启动子DNA的片段在枯草芽抱杆菌中的克隆

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摘要 用限制性核酸内切酶*Eco*R1酶切枯草芽抱杆菌168染色体DNA和pPL603质粒DNA然后用T4DNA连接酶连接，转化枯草芽抱杆菌BR151感受态细胞。在每毫升含有10微克氯霉素的SBPY选择培养皿上筛选，得到61个抗氯霉素的转化子。经快速琼脂塘凝胶电泳检测，从61个抗性转化子中得到49个比原载体pPL603质粒分子量大，并带有启动子DNA片段的重组质粒。测定了所有重组质粒表达的不同抗性水平，分析了部分质粒的一些特性，对抗性水平较高的7个重组质粒的分子量进行了测定，并用pBP61重组质粒DNA进行了第二次转化和酶切电泳分析。

关键词

分类号

Cloning Promoter Containing DNA Restriction Fragment in Bacillus subtilis

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Abstract

Plasmid pPL603(3.1Md) containing a structure gene for chloramphenicol acetyltransferase(Cat). Cells harboring the plasmid cannot grow on solid media containing 10ug/ml of chloramphenicol. Cloning EcoRI generated fragments of DNA into site of EcoRI in plasmid pPL603, withsubsequent selection of transformants of media containing 10ug/ml of chloramphenicol, permits the identification of restricion fragments that promote expression of the Cat gene Bacillus subtilis 168 chromosomal DNA and pPL603 DNA were digested with restriction endonuclase EcoRI and were ligated with T4 DNA ligase. B.subtilis BR151 competent cells were transformed by these recombinant plasmids. The transformants were selected on SBPY plates containing 10ug/ml of chloramphenicol. Forty nine recombinant plasmids with molecular weights were larger than pPL603 plasmid were found by rapid agarose gel electrophoresis. All these recombinant plasmids promoted the expression of the Cat gene.The different levels of chloramphenicol resistance of these recombinant plasmids were tested. The molecular weights of seven of these recombinant with highen level of chloramphenicol acetyltranse were further measured and properties of these recombinant plsmids analysed.

Key words

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