遗传学报　1981 8 (3): 189-195　ISSN: 0379-4172　CN: 11-5450/R

转座子Tn233（CH）分子量的测定

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收稿日期　修回日期　网络版发布日期　接受日期

摘要　转座子Tn233(CH)带有str sul抗性基因，最早是在痢疾杆菌的抗药质粒DR233（Tcr Cmr Smr Sur ）中发现的。现在通过菌株间的配对，将插入了Tn233(CH)转座子的质粒R144did3::Tn233(CH)转移到E.coliC600/PBR322(Apr、Tcr)细胞中，组成两种质糕点共存的菌株。从此菌株中提取出质粒DNA，用转化方法使它转移到E.coliC600/pBR322（Apr、Tcr）细胞中，组成两种质粒共存的菌株。从此菌株中提取出质粒DNA，用转化方法使它转移到E.coliC600菌株，再从所得到的转化子中用复印方法筛选出Tn233（CH）转座到pBR322质粒的转化子E.coli C600/pBR322::Tn233(CH),然后提出此质粒DNA，经限制性内切酶BamHI、EcoRI、PstI、HindIII与PvuII等酶切后，在琼脂糖凝胶平板与聚丙烯酰胺凝胶柱上进行电泳分析，分别以BamHI 与EcoRI双重酶解的λDNA、HindIII 酶解的T5DNA、HacIII酶解的M13 DNA与HacIII酶解的pBE322 DNA 作为泳动的标记，计算出质粒酶解片段的分子量，用此方法算出各片段分子量的总和为15.93×106道尔顿，此即为所求的pBR322::Tn233(CH)分子量，将此值减速去pBR322的分子量2.87×106道尔顿，得到Tn233（CH）的分子量为13.06×106道尔顿。电泳结果还表明在Tn233（CH）DNA分子上，BamHI、EcoRI、PstI、HindIII与PvuII分别有5、9、1、6、2个切点数。

关键词

分类号

Molecular Weight Determination of Transposon Tn233(CH)

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Abstract

Transposon Tn 233 (CH) contains str sul-resistant genes which are originally located to drug-resistant plasmid DR233 (Ter Cmr Smr Sur)harbored by Shigella flexneri strain 233. Plasmid R144drd3::Tn233 (CH) was constructed previously by transposition of Tn233 (CH) from DR233 (Kmr) and was then transferred to E. coli C600/pBR322 (Apr Tcr), so that strain with two coexisting plasmids was constructed. The plasmid DNA prepared from this coexisting strain was used to transform E. coli C600 cells and was lelected for Te resistant transformants on L-broth plate containing 12.5μg Te pre ml. After the transformants had grown on Te containing plates, E. coli C600/pBR322::Tn233 (CH)strain was sleected from these colonies on the same medium containing 12.5μg/ml Sm by replica method. Then plasmid DNA was extracted from E. coli C600/pBR322::Tn233 (CH) cells. The purified plasmid DNA preparations were digested by restriction endonuclease BamHI, EcoRI, PstI, HindⅢ, PvuⅡ and subjected to electrophoresis through agarose horixontal gel slabs and polyacrylamid gel in Tris-Acetate or Loening bouffer using DNA digested by both bamHi and EcoRI, T5 DNA digested by HindIII and M13 DNA digested by HaeIII as mobility markers. The molecular weight of these DNA fragments were measured. The molecular weight of plasmid pBR322::Tn233 (CH) calculated by summation of all the bands to be of approximately 15.93×106daltons, assuming that there is no deletion on pBR322during the transposition experiment. If we take pBR322 as 2.87×106 daltons, the molecular weight of Tn233 (CH) is 13.06×106 daltons. The electrphoresis results also indicated that the number of substrate site of restriction endonuclease BamHI, EcoRI, PstI, HindIII and PvuII on the Tn233 (CH) DNA sequence were 5,9,1,6 and 2 respectively.

Key words

DOI:

通讯作者