

利用转座子Tn233(CH) 与Tn5作为基因载体的研究

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摘要 用体外重组技术构建了转座子Tn233(CH), Tn233AE14与Tn5的3个衍生物:(1) 转座子Tn 2 3 3 (CH) 含有单个BglIII限制位点, 将从质粒pTK 6 3来的含K88. 抗原基因的BamHI片段连接到pBR322: :Tn233(CH) 的BglII位点上, 形成了pBR322::Tn233(K88)。(2) Tn233oE14是Tn233的缺失变种, 此缺失除去了Tn233(CH) 上的TnpA基因, 但保留了BglIII位点, 将同上面一样的BamHI片段克隆到pBR322: : Tn233AE14的BglIII位点上, 构建了pBR322: :Tn233AE14(K88)。(3)Tn5含有1个BamHI限制位点, PTB341是1个有Tn5插入的质粒, pTB341经BamHI切割后得到3个BamHI片段, 每个片段分别带有Apr基因; IS50L与Kin"基因; IS50R与Sin,基因, 当此3个片段经T4-DNA连接酶重新连接后, 分离到了1个质粒pTS40, 此质粒也由此3片段组成, 但它们的排列与PTB341的不同, 此重新连接的结果使Apr基因成为Tn5中的一部分。

遗传实验结果表明, K88抗原基因与Apr基因在这些转座子衍生物中能够表达, 而且新构建的Tn233(K8)与Tn5 (Ap) 仍保留着转座的能力。当在反式位置上有TnpA基因时, Tn233AF14(K8S) 也能从pBR322: :Tn233AE14(KS8) 转座到其它质粒上。

关键词

分类号

Studies on Utilization of Transposons Tn 233(CH) and Tn5 as Gene Vectors

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Abstract

Derivatives of transposons Tn233 (CH) , Tn233AE14 and Tn5 wore constructed using in vitro. recombination techniques. (1) Transposon Tn233 (CH) has a unique Bg11I restriction site, a BamHI fragment containing K88 antigen genes generated from plasmid pTK63 was ligated to this Bg11I- site o,f pBR322: :Tn233 (CH) to form pBR,322: :Tn233 (K8S) . (2) Tn233AE14 }is a deleti.ozimutant of Tn233 (CH); the deletion removed the TnpA gene of Tn233 (CH) , but retained the Bg111 site. The ;game BamIII fragm. ent was eltoned into the Bgl11 site of pBR322: :Tn233AE14 to construct pBR322 :Tn233AE14 (K88) . (3) Tn5 h. 、 a unique BantHI restriction site, and PTB341 is a Tn5 inserted plasmid. Cleavage of pTB341. with BamHI yields-three BamHI frag-3-Ments, with. fragments containing Apr gene, IS50L and Kntr gene, IS50R and 1Jmr gene, respectively. When these fragments were re-ligated with T4-DNA ligase, a plasmid pTS40 was isolated, which also consists of these fragments, but their arr-,ingement differs with that of pTB341. The consequence of re-ligation is that the, A pr gene becomes a. portion of a Tn5,
 The results of genetic experiments are able to expres themselves in these cted Tn23 (K8) and Tn5 (Ap) retain (K8) is also able to transpose from pB the presence of a wild type TnpA giune.sho'w that the K88 antigen Genes and Apr gene derivatives of transposons, and the new construed their transposition ability. The Tn233A14 8.322: :Tn2330E14 (.K88) to other plasiuid in the presence of a wild type TnpA gene.

Key words

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扩展功能

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