遗传 2006 28 (2): 212-218 ISSN: 0379-4172 CN: 11-5450/R

技术与方法

一种通用高效的复杂载体构建的新方法

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收稿日期 2005-3-11 修回日期 2005-5-6 网络版发布日期 2006-2-16 接受日期

摘要

文章报道了一种简便、通用、高效的复杂载体构建方法。此法是在PCR引物设计时，在目的片段5′端加上随机设计的接头，利用PCR克隆目的片段，再用T4 DNA聚合酶3′→5′外切酶活性处理PCR克隆目的片段，产生多个首尾相匹配的粘性末端，进行多片段定向连接、转化、重组子鉴定。以7片段拼接的水稻单交换质体定点整合表达载体pRSMGA的构建为例，应用上述方法构建只需做两次连接、转化，且其重组、转化效率高。数10次实验证明：这是一种简便、通用、高效的复杂构建载体的方法，该法至今尚未见报道。

关键词 复杂载体构建；新方法；T4DNA聚合酶；水稻

分类号 Q78

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A Universal High-throughput Novel Method of Constructing the Vectors

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Abstract

In<B> </B>this paper, a simple universal high-throughput method of constructing the vectors was developed. It could add proper adapter when the PCR primers were designed, and the purpose fragments were cloned by PCR, and the various complementary sticky ends were created by T4DNA polymerase's 3′-exodeoxyribonuclease activity. If all these fragments were put together with DNA ligase, they would recombinate in an orientation. If they had been transformated, the tansformants would be identified. Let's take the <I>Oryza sativa</I> single-cross homologous recombination chloroplast expression vector pRSMGA which was constructed with seven fragments as an example, if the vector pRSMGA was constructed in using the method what had mentioned, only twice recombination and transformation would be done. Scores of experiments had proved that it is a simple universal high-throughput novel method to construct the complicated vectors, which has not appeared in the periodical.

Key words constructing the complicated vectors novel method T4 DNA polymerase *Oryza sativa*

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