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研究报告

Red/ET重组在基因打靶载体快速构建中的应用

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摘要 通过合理应用Red/ET重组技术实现基因打靶载体的快速构建。在Red/ET重组介导下，首先从基因组DNA中将靶基因片段亚克隆至打靶质粒载体中，随后将两端带有50 bp同源臂的抗性筛选基因插入并替换靶基因上的目标序列，如此两步操作即可完成一个传统型基因敲除打靶载体的构建；结合Cre-loxP系统，在传统型基因敲除打靶载体的基础上，经过再一轮的Red/ET重组就能够成功实现条件性基因敲除打靶载体的构建。整个实验过程不需要PCR扩增长、短臂序列，也不涉及酶切、连接反应，因此，不仅省时、省力，而且所构建的基因打靶载体序列准确，无突变。此实验方法的建立为加速后基因组时代的基因功能研究提供了一条捷径。

关键词 基因打靶载体构建；Red/ET重组；基因敲除

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The Application of Red/ET Recombination to High Efficient Gene-targeting Vector Construction

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Abstract

A rapid and high efficient working system for gene-targeting vector construction was developed by using Red/ET recombination. Mediated by Red/ET recombination, the objective genomic DNA was first subcloned into the targeting vector. After insertion of a PCR amplified selectable marker gene flanked with short homology arms into the targeted position, a conventional gene knock-out targeting vector was then constructed. For conditional gene knock-out targeting vector construction, with the co-operation of Cre-loxP site-specific recombination, two rounds of Red/ET recombination was just needed. Being different from PCR and endonuclease-based gene-targeting vector construction, the homologous regions used for gene targeting can be chosen as long as possible. Furthermore, no enzyme digestion, ligation and sequencing identification were involved, so that it is very efficient and labor-saving. Several different gene-targeting vectors were successfully constructed by using this system. The establishment of this working system will accelerate the gene function studies in the post-genome stage.

Key words gene-targeting vector construction Red/ET recombination gene knock-out

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