

<<转基因技术>>

图书基本信息

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内容概要

在后基因组时代，学界所面临的主要挑战之一是如何破解大量的编码蛋白质的基因功能。在《转基因技术(原理与实验方案原著第3版导读版)(精)》(作者卡特莱特)中，该领域的专家在第2版的基础上进行了更新和补充，以求能够详尽地反映当前基因修饰技术的最新进展。

《转基因技术(原理与实验方案原著第3版导读版)(精)》不仅包括基因修饰小鼠制作过程，同时也介绍了其他模式生物的转基因技术，以及在显微注射、位点特异性重组系统、冷冻保存等方面的探索和尝试。

本书秉承Springer《分子生物学方法》系列丛书的一贯风格，阐述明晰、便于使用，每章包括对相关问题的介绍，所需材料和试剂的清单，实验操作的具体步骤，以及常见问题的解决方法和缺陷规避。

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章节摘录

Summary Transgenesis in *Drosophila melanogaster* relies upon direct microinjection of embryos and subsequent crossing of surviving adults. The necessity of crossing single flies to screen for transgenic events limits the range of useful transgenesis techniques to those that have a very high frequency of integration, so that about 1 in 10 to 1 in 100 surviving adult flies carry a transgene. Until recently, only random P—element transgenesis fulfilled these criteria. However, recent advances have brought homologous recombination and site—directed integration up to and beyond this level of efficiency. For all transgenesis techniques in *Drosophila melanogaster*, microinjection of embryos is the central procedure. This chapter gives a detailed protocol for microinjection, and aims to enable the reader to use it for both site—directed integration and for P—element transgenesis.

Key words: *Drosophila melanogaster*, Embryo, Microinjection, Transgenic, Recombination, Integration, Homologous recombination, phiC31 / integrase, Site—directed integration, p—element

1. Introduction Transgenesis in *Drosophila melanogaster* has undergone something of a revolution in the last few years. The classical technique of random P—element—mediated transgenesis has recently been supplemented by two novel technologies: homologous recombination and C31 integration (for reviews, see (1) and (2)). In P—element transgenesis (3), a modified transposon vector is used in combination with transient expression of the P transposase enzyme to generate several fly lines, with different insertion sites in the genome. These insertions are subsequently mapped and characterised. P—element insertions have been invaluable for mutagenesis screens, but until recently, this was also the only method available for introducing a transgene of choice into the *Drosophila* genome. The random nature of P—element insertions has several drawbacks for transgene analysis. Mapping of insertion sites is time consuming, and transgene expression levels are subject to genomic position effects, making it difficult to draw comparisons between different constructs.

A recently developed alternative to random insertion is homologous recombination (4, 5). This involves inserting a donor construct at random into the genome by P—element transgenesis, and in subsequent generations, mobilising the donor construct to the correct locus by homologous recombination. This technique had long been lacking to *Drosophilists*, but has not replaced P—element transgenesis as the method of choice for routine transgene analysis, because both the cloning of donor constructs and the generation of homologous recombinants are more time consuming than for P—element transgenesis. Recently, C31 integration has been developed (6). This technique allows rapid and efficient generation of site—specific integrants, and relies upon 'docking site' fly lines, which carry a single recognition site (attP) for the phage C31 integrase enzyme, previously introduced into the genome by P—element transgenesis. A donor plasmid carrying a second recognition site (attB) and a source of integrase enzyme is used to generate those in which the donor plasmid docks to the genomic site. Integration events are highly specific, as the attP site is 39 bp long and does not occur at random in the *Drosophila* genome. Many mapped and characterised docking site lines are now available (see Note 1), and C31 integration is rapidly becoming widely used for many transgenic applications.

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编辑推荐

《转基因技术：原理与实验方案（实验室解决方案）（原著第3版）》特点： 本书为Springer经典实验室指南系列《分子生物学方法》（Methods in Molecular Biology）的分卷。

第3版对制备转基因动物的实验室可提供全面指导，对进行与转基因相关科学研究的实验室亦可提供丰富、宝贵的知识和信息。

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