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转基因技术

原理与实验方案（原著第3版）

Transgenesis Techniques

Principles and Protocols (Third Edition)

Elizabeth J. Cartwright

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导　读

一提起转基因，人们首先想到的可能就是转基因食品。随着转基因食品的安全问题日益成为争议的焦点，转基因这一名词也越来越多地出现在人们的视野里，与我们的日常生活日益相关。所谓的转基因就是将经过人工分离和改造过的基因片段通过一定的方式方法转入到特定的生物体内，从而使生物体表现出特定性状的过程。转基因最初用于科学研究，旨在揭示基因的功能。转基因技术的快速发展和成熟得益于限制性内切酶的发现，正是由于限制性内切酶的发现，才使得人们能够在体外可以随心所欲地对 DNA 进行改造和修饰，自由地对 DNA 进行转移。自第一只转基因小鼠于 20 世纪 80 年代出现之后，各种基因修饰的小鼠相继涌现，使得人们对于基因功能的了解更上层楼。

随着转基因技术的不断发展和成熟，转基因在农业和畜牧业中的应用也越来越广泛。通过将生长激素、高泌乳量基因、瘦肉型基因及抗病毒基因等转入生物个体内，人们已经可以轻而易举地获得生长周期短、泌乳量高、具有抗病性的动物；培育出了高产、抵抗病虫害、抗除草剂等的作物新品种；利用转基因动物作为生物工厂，如输卵管生物反应器、乳腺生物反应器等，生产人血红蛋白、凝血因子、白介素等，极大地促进了生物医药产业的发展。尽管转基因食品的安全问题、转基因生物对生态环境和生物多样性的影响一直存在着很大的争议，但是转基因技术对于科学研究和生物医药等领域所起到的巨大推动作用是毋庸置疑的。

事实上，自从人类历史上开始耕种作物和饲养家畜以来，对作物和家畜的遗传改良就从来没有停止过。千百年来，我们的祖先就通过对自然突变产生的优良基因进行选择和利用，采用人工杂交的方法对优良基因进行重组和积累以达到优选优育的目的。遗传与变异，作为生物界不断地普遍发生的现象，正是物种形成和生物进化的生物学基础。而今蓬勃发展的转基因技术与传统的技术事实上是一脉相承的，本质上都是对生物进行遗传改造和修饰。转基因技术是在遗传学、分子生物学的基础上，与传统技术相结合发展起来的。与传统的改良技术相比，转基因技术更为高效，可以大规模地操作；可以不受生物体间亲缘关系的限制、打破生物界物种之间的界限，在不同的物种之间进行基因转换，而不像传统技术那样，只能在物种之内不同的生物个体之间进行基因转移。转基因另外一个显著的特点是在分子水平进行改造和修饰，针对的是特定、明确的基因片段，可以较为准确地预测子代的性状和表型，而不像传统技术那样无法对特定基因进行操作和选择，是在生物个体水平上进行的。传统技术转移的不仅仅是所希望的候选性状/基因，同时还带有包含其他大量无关基因的基因组，因而对于后代性状的预测性是较弱的。因此，转基因技术是对传统技术的继承和发展。

工欲善其事，必先利其器。在体研究基因的功能，是我们理解生物胚胎发育、基因生理功能、疾病发生发展机理乃至生物进化的基础。通过基因修饰，人们可以深入研究目的基因在胚胎发育过程中的作用，探讨其作用机制；根据临床线索对基因进行突变，建立与人类疾病具有分子细胞机理同源性、行为表型一致性的动物模型，不仅可以贴切真实地反应疾病的发生和发展过程，明确疾病的发生机理，并且有助于疾病的早期诊断

及治疗药物的开发。因此，现代转基因技术无疑是科学研究领域非常强大的工具和手段。本书不仅适合于转基因领域的专业人士阅读和参考，也同样可加深从事与转基因领域相关的科研人员，包括教师、研究生和高年级本科生对这一领域的认识。书中汇总了转基因领域内专家们的经验积累和心得，将为从事和即将从事相关研究的科研人员指点迷津。

赵春杰

2012 年春于南京

前　言

当人类基因组序列全部被破解之后，学界所面临的主要挑战之一就是如何明确20,000-25,000编码蛋白质的基因的功能。这本书对目前所用在体研究基因功能的基因修饰技术做了详细的介绍。

得益于转基因技术，小鼠迄今为止已成为研究哺乳动物基因功能最受欢迎的模式动物，这不仅是因为小鼠的基因组与人类较为相近，而且由于小鼠的生理特征、解剖结构在某种程度上也与人类相似。虽然本书大部分的篇幅主要是介绍小鼠的转基因技术，但小鼠并非唯一的模式生物。其他一些模式动物如果蝇、线虫、爪蟾、斑马鱼和大鼠，在胚胎发育、基因的生理功能及人类疾病基因的功能研究中都发挥着非常重要的作用，在转基因研究中也常用到。本书中对于以上每种模式动物的转基因，都专门安排了一个章节进行介绍，详细介绍了动物的繁殖、构建载体、基因转入等，在介绍经典转基因技术的同时也介绍了最新发展起来的方法。

本书用大部分的章节介绍了通过将外源DNA注射入受精卵的前核和基于在胚胎干细胞进行同源重组打靶技术的基因修饰小鼠的制作，该部分内容是专门为这本新版《转基因技术：原理与实验方案》编写的，涵盖了该领域内的最新进展。主要介绍了构建转基因载体的最佳方案和策略、详尽的前核显微注射技术及相关的外科手术操作、维持胚胎干细胞多能性的最佳培养条件以及打靶技术等。第13-15章重点阐述了如何通过将打靶后的胚胎干细胞注射入囊胚或在桑葚胚阶段通过细胞聚集获得嵌合体小鼠的技术手段、将改造后的胚胎重新转入代孕母鼠体内的外科手术。多年来，Cre/loxP和flp/frt重组系统一直很受人们的欢迎，在16章对这两个系统及其在小鼠基因组修饰操作中的应用进行了介绍。17章重点介绍了Cre转基因小鼠的制作和应用。另有一章介绍了大规模的国际间合作以求在基因组中系统性敲除每个基因的工作。本书的其他章节介绍了成功增殖转基因小鼠所必备的饲养和繁殖技巧、冰冻保存以及从冰冻状态下复苏小鼠的技术。

这是一本全面的制作转基因动物的实用手册，同时汇集了该领域专家在具体实践过程中日积月累的心得体会，这些宝贵的经验对于目前正在进行转基因研究的实验室和即将进入该领域的初涉者来说是无价的。本书也为使用（但并不制备）转基因动物模型的科学家们提供了重要的背景信息。

就我个人来说，非常高兴能够主编最新版的《转基因技术：原理与实验方案》。首先，通过通读以前的版本，收获颇丰，也希望新版本对其他人能够有所帮助和激励。其次，能与转基因领域的专家们一起编写此书，本人深感荣幸。

伊丽莎白·J·卡特莱特

曼彻斯特，英国

（赵春杰　译）

To Dan, Edward and William with love.

Preface

One of the major challenges currently facing the scientific community is to understand the function of the 20,000–25,000 protein-coding genes that were revealed when the human genome was fully sequenced. This book details the transgenic techniques that are currently used to modify the genome in order to extend our understanding of the in vivo function of these genes.

Since the advent of transgenic technologies, the mouse has become by far the most popular model in which to study mammalian gene function. This is due to not only its genetic similarity to humans but also its physiological and, to a certain extent, its anatomical similarities. Whilst a large proportion of this book is dedicated to the use of the mouse in transgenesis, the mouse is certainly not the only model to provide essential information regarding gene function. A number of other valuable models are used in transgenic studies including *Drosophila*, *C. elegans*, *Xenopus*, zebrafish, and rat. For each of these species, a chapter in this book is dedicated to highlighting how each is particularly suited, for example, to the study of embryonic development, physiological function of genes and to study orthologs of human disease genes. These chapters give detailed practical descriptions of animal production, construct design, and gene transfer techniques; recently developed methods will be described along with highly established classical techniques.

A number of chapters in this book are dedicated to the generation of genetically modified mice by the present classic techniques of injection of exogenous DNA into the pronuclei of fertilised eggs and by gene targeting using homologous recombination in embryonic stem cells. These chapters, as with all the others in the book, have been specifically written for this edition of *Transgenesis* and so contain up-to-date details of the practices in the field. Chapters are included describing optimal transgene and construct design, in-depth technical details for pronuclear microinjection of transgenes and associated surgical techniques, details for the optimal conditions in which to culture embryonic stem cells in order to maintain their pluripotent state, and methods for targeting these cells. A combination of chapters (Chaps. 13–15) describe how to generate chimaeras by microinjection of targeted ES cells into blastocysts or by morula aggregation, and the surgical techniques required to transfer the resulting embryos. For a number of years, the use of Cre/loxP and flp/frt recombination systems has gained in popularity; Chap. 16 describes their use and introduces other state-of-the-art site-specific recombination systems that can be used to manipulate the mouse genome. The generation and use of Cre-expressing transgenic lines are described in Chap. 17. One chapter of the book highlights the large-scale international efforts that are being made to systematically knockout every gene in the genome. The remaining chapters detail the breeding and husbandry skills required to successfully propagate a transgenic line and the increasingly essential methods for cryopreserving a mouse line and recovering lines from frozen stocks.

This book is a comprehensive practical guide to the generation of transgenic animals and is packed full of handy hints and tips from the experts who use these techniques on a

day-to-day basis. It is designed to become an invaluable source of information in any lab currently involved in transgenic techniques, as well as for researchers who are newcomers to the field. This book also provides essential background information for scientists who work with these models but have not been involved in their generation.

On a personal note, it has been a great pleasure to edit this latest edition of *Transgenesis*. Firstly, I learnt many of my skills from reading earlier editions of the book and I hope that this edition will help and inspire many others. Secondly, I have been privileged to work with the exceptionally talented researchers in the transgenesis field who have contributed to this book.

Manchester, UK *Elizabeth J. Cartwright*

Contributors

IGNACIO ANEGON • *INSERM – Institut National de la Santé et de la Recherche Médicale, Nantes, France*

J. SIMON C. ARTHUR • *MRC Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee, UK*

KAZUHIDE ASAKAWA • *Division of Molecular and Developmental Genetics, National Institute of Genetics, Mishima, Shizuoka, Japan*

IVANA BARBARIC • *Department of Biomedical Science, University of Sheffield, Sheffield, UK*

MARIE-CHRISTINE BIRLING • *Institut Clinique de la Souris – Mouse Clinical Institute (ICS-MCI), Illkirch, France*

VALÉRIE BRAUN • *genOway SA, Lyon, France*

ELIZABETH J. CARTWRIGHT • *Cardiovascular Medicine, University of Manchester, Manchester, UK*

YACINE CHERIFI • *genOway SA, Lyon, France*

JEAN COZZI • *genOway SA, Lyon, France*

T. NEIL DEAR • *Leeds Institute of Molecular Medicine, St. James's University Hospital, Leeds, UK*

MARTIN FRAY • *Frozen Embryo & Sperm Archive (FESA), Medical Research Council, Mammalian Genetics Unit, Harwell, UK*

ROLAND H. FRIEDEL • *Institute of Developmental Genetics, Helmholtz Center Munich, Neuherberg, Germany*

WENDY J.K. GARDINER • *Mary Lyon Centre, Medical Research Council, Harwell, UK*

FRANÇOISE GOFFLOT • *Institut Clinique de la Souris – Mouse Clinical Institute (ICS-MCI), Illkirch, France*

ANNE-CATHERINE GROSS • *genOway SA, Lyon, France*

JAMES GULICK • *Molecular Cardiovascular Biology, Cincinnati Children's Hospital, University of Cincinnati, Cincinnati, OH, USA*

KOICHI KAWAKAMI • *Division of Molecular and Developmental Genetics, National Institute of Genetics, Mishima, Shizuoka, Japan*

HIROSHI KIKUTA • *Division of Molecular and Developmental Genetics, National Institute of Genetics, Mishima, Shizuoka, Japan*

CHRISTIAN KLASEN • *Transgenic Service, European Molecular Biology Laboratory, Heidelberg, Germany*

NIKOS KOURTIS • *Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece*

JANA LOEBER • *Department of Developmental Biochemistry, University of Goettingen, Goettingen, Germany*

VICTORIA A. MCGUIRE • *MRC Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee, UK*

CHRISTEL MERROUCHE • *genOway SA, Lyon, France*

FONG CHENG PAN • *Vanderbilt University Program in Developmental Biology and Department of Cell and Biology, Vanderbilt University Medical Center, Nashville, TN, USA*

ANGELA PASPARAKI • *Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece*

TOMAS PIELER • *Department of Developmental Biochemistry, University of Goettingen, Goettingen, Germany*

ANNE PLÜCK • *Centre for Mouse Genetics, Institute for Genetics, University of Cologne, Cologne, Germany*

MATTHIAS RIECKHER • *Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece*

LEONIE RINGROSE • *IMBA – Institute of Molecular Biotechnology GmbH, Vienna, Austria*

JEFFREY ROBBINS • *Molecular Cardiovascular Biology, Cincinnati Children's Hospital, University of Cincinnati, Cincinnati, OH, USA*

KAI SCHUH • *Institute of Physiology I, University of Wuerzburg, Wuerzburg, Germany*

MAXIMILIANO L. SUSTER • *Division of Molecular and Developmental Genetics, National Institute of Genetics, Mishima, Shizuoka, Japan*

NEKTARIOS TAVERNARAKIS • *Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece*

LYDIA TEBOUL • *Mary Lyon Centre, Medical Research Council, Harwell, UK*

MELANIE ULLRICH • *Institute of Physiology I, University of Wuerzburg, Wuerzburg, Germany*

AKIHIRO URASAKI • *Division of Molecular and Developmental Genetics, National Institute of Genetics, Mishima, Shizuoka, Japan*

LUCIE VIZOR • *Medical Research Council, Harwell, UK*

XIN WANG • *Faculty of Life Sciences, University of Manchester, Manchester, UK*

XAVIER WAROT • *EPFL FSV – École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland*

SARA WELLS • *Medical Research Council, Harwell, UK*

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（赵春杰　译）

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Part I

Transgenesis in Various Model Systems

Chapter 1

Transgenesis in *Drosophila melanogaster*

Leonie Ringrose

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 ΦC31 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

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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, ΦC31 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 ΦC31 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 flies 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 ΦC31 integration is rapidly becoming widely used for many transgenic applications.

All these transgenic techniques rely upon microinjection of embryos as a first step. In early *Drosophila* embryogenesis, the nuclei share a common cytoplasm for the first nine divisions. Directly after the tenth division, the first cells to become separated are the pole cells, which will later form the adult germ line. Transgenic animals are made by microinjecting DNA and a source of enzyme (P-transposase or ΦC31 integrase, *see* **Note 2**) into the posterior of the embryo where the pole cells will form, at an early stage before they have become separated from the common cytoplasm. DNA can enter the nuclei and is integrated into the genome of some cells. Embryos are allowed to mature and the adults are outcrossed to screen for transgenic flies in the next generation.

This chapter gives a detailed description of microinjection, from preparing DNA to screening for transformants. The main protocol deals with ΦC31 integration as we perform it in our laboratory. Alternatives for both ΦC31 and P element transgenesis are given in the notes.