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基因敲除实验指南

（原著第2版）

Gene Knockout Protocols

(Second Edition)

Ralf Kühn and Wolfgang Wurst

科学出版社

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导　读

由 Ralf Kühn 和 Wolfgang Wurst 等人编著的《基因敲除实验指南》第二版在第一版的基础上吸收更新了近年的新技术成果，全书四部分共二十五个章节全面叙述了关于小鼠基因定点敲除敲入的实用技术体系，是一本系统介绍动物转基因技术的好书。本书内容涉及当前国际上动物基因敲除技术的方方面面，提供了一个关于 Gene Knockout 发展现状的全景视窗。对于中国这样一个在生命科学领域全面追赶国际先进水平的国度，本书导读版的引进会对我国相关研究领域的技术普及提供一个强劲的推动，同时为专业科技人员及研究生的教学科研提供一个权威参考。作为面向生命科技工作者的技术工具类书籍，本书具有以下几个特点。

第一，在小鼠基因敲除技术方面的描述具有高度的科学权威性。本书大多数作者是在小鼠遗传学相关领域做出重要成果并在国际专业杂志发表了高质量科技论文、对基因敲除技术有具体贡献的欧美专家和学者。科学的重要特征之一是其结果能被他人重复。出自权威学者的实验步骤、程序和结论，其科学性和可靠性大为提高，有利于科学技术的普及。这些专家学者不仅自身经验丰富，又热心基因敲除技术的传播，使再版的专著有利于提高读者的学习效率，学到真东西和好东西。

第二，所描述的基因操作技术具有实用性和可操作性。细心的读者会发现，无论从第一部分的干细胞技术到细小的显微操作仪操作，本书秉承了优秀科技书籍在叙述方面具体和细致的特点，特别是程序及步骤描写的实用性和可操作性很强，甚至在一些时间节点安排上都有必要的标示和建议，图文并茂，基本不留死角，更不搞“留一手”，使得学习者比较容易理解和掌握关键技术和技巧。只要学习操作者充分准备，严格按要求操作，就比较容易得到预期的结果。这种把自身宝贵的实践经验体现在全面细致的操作描述方面本身就体现了科学精神，对于先进科学技术的普及特别重要。实用和可操作的另一个体现是读者可以各取所需，只阅读和关注自己感兴趣的章节。

第三，各章节对所涉及技术都列出了相对全面的文献参考。几乎任何一项高科技技术的发展都经历了一个概念、发生、发展、成熟和优化的过程。今天我们看到的一个具体程序或流程的描述往往是许多科学工作者在一定时期内智慧和创新的凝结与积累，并在实践中不断改进、优化而走向新的高度。全面列出一项技术发展过程中的关键文献有利于研究人员结合自身和科研对象特点深入了解和学习相关历史或来龙去脉，为技术进一步提升发展展现宽广基础和可能性，为可能的创新提供平台。这些文献虽为英文，还是为感兴趣的读者提供了第一手参考资料。

第四，描述语言在客观准确的基础上相对活泼丰富。这可能是从事小鼠基因功能研究的科学家的多元性造成的。过去几十年来从北美到欧洲，几乎所有从事生命科学特别是人类疾病及基因功能研究的重要大学及研究机构都不同程度开展了平行独立的小鼠基因敲除研究。科学技术有其内在规定性，在满足了对科技描述客观准确的基本要求的基础上，一定的个性化是客观存在并允许的。

基因功能的研究是一项长期而重大的科学任务。地球上所有生命的同源性使得我们可

以通过小鼠等模式生物的基因研究来帮助理解人类同源基因的功能，为人类健康长寿和开发遗传资源提供科学知识。人类基因的功能一般无法直接用人体做材料来研究，其知识几乎都是通过对小鼠等模式生物的研究而得到的。然而到目前为止，全球范围内小鼠基因敲除模型还不足4000种，不到已知基因总数量的四分之一，研究工作任重而道远。更有甚者，多因子复杂疾病基因的功能研究目前还没有很好的方法。具体到中国，由于我们早期科技投入、知识及人才积累和语言文化等因素，通过基因敲除进行小鼠基因功能的研究尚未在国内得到普及，这实际上是导致我国生命科学技术落后于欧美发达国家的主要原因之一。正因为此，本书的引进显现出巨大的科学传播价值。

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前　言

在小鼠和人类基因组测序完成后，解读每一个基因功能特性及其分子间作用网络就成为巨大挑战。小鼠作为模式动物为人类生物学和疾病的遗传研究做出了其他哺乳动物无可比拟的贡献。小鼠的发育、身体结构、生理机能、行为方式和疾病特征与人类有很多共同性，事实上，99%的人类基因与小鼠基因同源。在马里奥（Mario Capecchi）和他的同事首次报道了小鼠干细胞进行基因打靶后，利用小鼠模型来研究基因功能的技术已经有了长足发展，二十多年来，科研工作者已经制备了超过3000种基因突变小鼠。迄今为止，各种修饰基因或修饰表型的小鼠基因突变技术被系统地用于研究。基因组序列的获得使研究者能够高效而又精准地修饰基因，例如，在胚胎干细胞中进行基因捕获和定向突变等，结合位点特异性DNA重组技术，特别是Cre/loxP系统，可实现条件性突变小鼠的特定细胞中定向打断基因。对鼠基因组的化学突变和转座子突变进行表型筛选，可以识别与人类疾病模型有关的表型-基因型的相互关系。在未来几年里，系统性改变小鼠基因组和预定操作技术将会不断发展并进一步地完善。

《基因敲除实验指南》第二版整合了基因打靶和小鼠遗传学领域研究的突出贡献以及延伸而来的众多经验教训。本书沿袭了《分子生物学方法》系列丛书的成功模式，提供了完整、详细、既适用于初学者又适用于资深专家的实验操作指南。新版不止强调了过去七年里新的基因突变技术的发展，也囊括了经典基因打靶技术的基本方法。本书共25章分为四个部分，分别介绍了胚胎干细胞基因诱变、干细胞操作、基因工程小鼠的制备和突变体表型分析。这部分内容反映了现今实现基因突变方法的多样性，有经典的基因打靶技术、寡核苷酸修饰技术、突变基因捕获技术、RNAi介导的基因沉默技术、转座子和ENU突变技术等。在介绍通过基因打靶、基因捕获、基因沉默、基因组工程等方法构建条件性敲除载体以及构建和诱导Cre转基因小鼠的制备、Cre小鼠品系数据库这两章中，还包括了利用Cre/loxP重组系统实现条件性基因失活的内容。在大篇幅介绍产生新的突变体或转基因小鼠方法的同时，还完善了突变体的制备及突变表型相关的技术内容，包括精子冷藏、胚胎干细胞系的构建、干细胞体外分化、小鼠病理学、突变表型和表型遗传背景的影响。

我们希望专家撰写的新版《基因敲除实验指南》提供的一系列独特的实验操作技术，可以成为该领域学者们的宝贵资源，并进一步促进小鼠遗传学的研究。

Ralf Kühn

Wolfgang Wurst

（陈龙欣　马润林　译）

Preface

Following the completion of the mouse and human genome sequences, a major challenge is the functional characterization of every mammalian gene and the deciphering of their molecular interaction network. The mouse offers many advantages for the use of genetics to study human biology and disease, unmatched among other mammals. Its development, body plan, physiology, behavior, and diseases have much in common, based on the fact that 99% of the human genes have a mouse ortholog. The investigation of gene function using mouse models is based on many years of technological development. In the two decades since gene targeting in murine embryonic stem (ES) cells was first described by Mario Capecchi and colleagues, more than 3000 predesigned mouse mutants have been developed. To date, a variety of mouse mutagenesis techniques, either gene- or phenotype-driven, are used as systematic approaches. The availability of the genome sequence supports gene-driven approaches such as gene-trap and targeted mutagenesis in ES cells, allowing efficient and precise gene disruption. In combination with the use of site-specific DNA recombinases, in particular the Cre/loxP system, gene disruption can be directed to specific cell types in conditional mouse mutants. Furthermore, chemical and transposon mutagenesis of the mouse genome enables us to perform phenotype-driven screens for the unbiased identification of phenotype–genotype correlations involved in models of human disease. Over the next several years, the mouse genome will be systematically altered, and the techniques for achieving predesigned manipulations will be constantly developed further and improved.

The second edition of *Gene Knockout Protocols* brings together distinguished contributors with extensive experience in the gene targeting and mouse genetics fields. In line with the successful format of *Methods in Molecular Biology*, the volume provides a comprehensive collection of step-by-step protocols of use not only for the beginner in the field but also for experienced scientists. The new edition particularly emphasizes the range of new mutagenesis techniques developed over the last seven years, but also covers the basic methods relevant to researchers performing classical gene targeting experiments. The 25 chapters of this volume are organized into four sections on gene modification in ES cells, stem cell manipulation, the generation of genetically engineered mice, and mutant phenotype analysis. The contents reflect the diversification of mutagenesis approaches that now include, besides classical gene targeting, gene modification by oligonucleotides, gene trap mutagenesis, RNAi-mediated knockdown, transposon, and ENU mutagenesis. Conditional gene inactivation through Cre/loxP recombination is covered by chapters on the construction of conditional vectors for gene targeting, gene trap, gene knockdown, and chromosome engineering, complemented by chapters on the generation of constitutive and inducible Cre transgenic mice and the Cre mouse strain database. While most of the chapters describe methods to generate new mutants or transgenic mice the content is completed by techniques relevant for the preservation and phenotyping of mutants. These

include sperm freezing, ES cell line establishment, ES cell in vitro differentiation, mouse pathology, mutant phenotyping, and the influence of genetic background on phenotypes.

We hope that this new edition of *Gene Knockout Protocols* that provides a unique collection of bench protocols written by experts will be a valuable resource for all scientists in the field and will further stimulate research on mouse genetics.

Ralf Kühn
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Color Plates

Color Plate 1 Protocol for mES cell-derived cardiac differentiation. Five-day EBs were plated onto gelatin- or laminin-coated plates and cultured in IMDM+20%FCS supplemented with L-glutamine, NEAA, and MTG for up to 24 days. Multilineage progenitors at the intermediate stage 2 co-express nestin and desmin, while terminally differentiated cardiac clusters (stage 3) show well-organized sarcomeric staining of Z-disk epitopes of titin. Beating frequency measured from a beating cluster (phase contrast) by the LUCIA HEART imaging system is shown at the *right*, bar = 50 µm (*see* discussion on p. 229)

Color Plate 2 Protocol for mES cell-derived neuronal differentiation. ES cells were cultured as EBs for 4 days. After plating onto gelatin, cells were cultured in B1 supplements and FCS-containing medium for 24 h (*). After medium change (at day 4+1), EB outgrowths were cultured until day 4+8 without FCS to select for neural progenitors. At day 4+8, EBs were dissociated and replated onto poly-L-ornithine/laminin until day 4+14, when differentiation of mature neurons was induced by "Neurobasal" medium, B27 supplement, and SPFs ("survival promoting factors"). The table shows the media, additives, and substrates used with this protocol. Differentiation led to nestin-positive neural progenitors (stage 2) followed by β-III-tubulin-expressing neuronal cells at stage 3 (4+14 d) and dopaminergic neurons expressing tyrosine hydroxylase at stage 4. A phase contrast picture shows the morphology of the ES cell-derived neurons at stage 4 (*right*) (*see* discussion on p. 230)

Color Plate 3 Protocol for mES cell-derived pancreatic differentiation. Scheme displays media, additives, and substrates used during the differentiation process. Five-day EBs were plated onto gelatin for spontaneous differentiation in IMDM containing 20% FCS, L-Glut, NEAA, and MTG. At day 5+9, EBs were dissociated and replated onto poly-L-ornithine/laminin and subjected to differentiation by adding the differentiation factors niacinamide (NA), laminin, insulin, sodium selenite, transferrin, progesterone, and putrescine (and FCS for 24 h after plating). After medium change (at day 5+10), differentiation was continued (without FCS) until day 5+28. During spontaneous differentiation, nestin/CK19 co-expressing multilineage progenitors were formed (stage 2). Directed differentiation resulted in C-peptide/nestin-positive committed progenitors (stage 3) and insulin/C-peptide co-expressing islet-like clusters (stage 4; images

from (68)). Morphology by phase contrast is shown (*right*) from (77). Cell nuclei were visualized by Hoechst 33342 (*blue*). Bars = 20 μm (*see* discussion on p. 231)

Color Plate 4 Protocol for mES cell-derived hepatic differentiation. Scheme displays media, additives, and substrates used during the differentiation process. Five-day EBs were plated onto gelatin for spontaneous differentiation in IMDM containing 20% FCS, L-Glut, NEAA, and MTG. At day 5+9, differentiation into the hepatic lineage was induced by dissociation of the EBs and replating onto collagen I. Cells were cultured in differentiation medium (HCM) containing 10% FCS until day 5+9+30. Spontaneous differentiation led to nestin/AFP-positive multilineage progenitors (stage 2). Differentiation resulted in albumin/AFP co-expressing committed progenitors at stage 3, and albumin- and AAT-positive, partially binucleated hepatocyte-like cells (stage 4, images from (53)) with cuboidal morphology (phase contrast, *right*, from (77)) at day 5+9+30. Cell nuclei were visualized by Hoechst 33342 (*blue*). Bars = 20 μm (*see* discussion on p. 232)

目　录

（陈龙欣　马润林　译）

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