

Analysing Gene Expression

A Handbook of

Methods Possibilities and Pitfalls

基因表达分析手册

——方法的实用性及其缺陷

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陈凡 方晓华 张方 等译



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· 北京 ·

图书在版编目 (CIP) 数据

基因表达分析手册——方法的实用性及其缺陷/[德] 洛克沃斯基 (Lorkowski, S.), [爱尔兰] 卡伦 (Cullen, P.) 编著; 陈凡, 方晓华, 张方等译. —北京: 化学工业出版社, 2005. 2

(生物实验室系列)

书名原文: Analysing Gene Expression: A Handbook of Methods, Possibilities and Pitfalls
ISBN 978-7-5025-6630-2

I. 基… II. ①洛…②卡…③陈…④方…⑤张… III. 基因表达-分析-手册 IV. Q753

中国版本图书馆 CIP 数据核字 (2005) 第 007624 号

Analysing Gene Expression: A Handbook of Methods, Possibilities and Pitfalls, /Edited by Stefan Lorkowski & Paul Cullen
ISBN 3-527-30488-6

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Authorized translation from the English language edition published by Wiley-VCH Verlag GmbH & Co. KGaA.

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责任编辑: 刘亚军 周 旭

装帧设计: 关 飞

责任校对: 宋 玮

出版发行: 化学工业出版社 生物·医药出版分社

(北京市东城区青年湖南街 13 号 邮政编码 100011)

印 刷: 北京永鑫印刷有限责任公司

装 订: 三河市万龙印装有限公司

787mm×1092mm 1/16 印张 42 彩插 4 字数 1047 千字 2008 年 10 月北京第 1 版第 1 次印刷

购书咨询: 010-64518888 (传真: 010-64519686) 售后服务: 010-64518899

网 址: <http://www.cip.com.cn>

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基因表达分析手册

——方法的实用性及其缺陷

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出版者的话

21 世纪是生命科学的世纪，这已成为人们的共识。

生命科学随着人类对自身和自然的认识、探索而萌芽，随着人类生产和科学实践的进步而发展。现代生命科学包括生物学、医学、农学等传统学科领域，以及生物学、生物技术与环境科学乃至社会科学等其他学科相互渗透、交叉而产生的新型学科体系。20 世纪后叶现代生物科学尤其是分子生物学取得了一系列突破性成就，使得生命科学在自然科学体系中的位置发生了革命性的变化，成为 21 世纪的带头学科。人们对生命科学也寄予了无限的期望，希望能够解决人类社会所面临的人口膨胀、资源匮乏、疾病危害、环境污染和生态破坏等一系列重大问题。

回顾生命科学的发展历程，实验技术一直起着非常重要的促进作用。如 17 世纪 Leeuwenhoek 等人发明并应用显微镜技术，直接催生了“细胞学说”的建立和发展；1973 年 Cohn 和 Boyer 完成了 DNA 体外重组实验，标志着基因工程的肇始；1988 年 Kary Mullis 发明的 PCR 技术甚至使生命科学产生了飞跃性的发展。可以说，生命科学无时无刻离不开实验，实验是开启神奇的生命王国大门的钥匙。没有实验技术的不断进步，也就没有生命科学今天的巨大发展；同时，生命科学的发展又对实验技术提出了更高的要求，进一步刺激了后者的不断进步。生命科学正是在“实验催生和验证着基础理论，理论指导和促进了实验技术”的不断循环中从必然王国走向自由王国。

工欲善其事，必先利其器。为了有助于生命科学工作者更多地了解相关实验技术和仪器设备，更好地设计实验方案，更有效地开展实验过程，更合理地处理实验结果，化工出版社组织出版了“生物实验室系列图书”。系列图书在整体规划的基础上，本着“经典、前沿、实用，理论与技术并重”的原则组织编写，分批出版。

在题材上，系列图书涵盖综合实验技术和单项实验技术两个方面。其中综合实验技术既有以实验目的为题，如“蛋白质化学分析技术”，内容纵向覆盖多项实验技术；也有以某一生命学科领域的综合实验技术为题，如“发酵工程实验技术”、“生物化学实验技术”等。而单项实验技术则以深入介绍某一专项技术及其应用为主，在阐述其基本原理的基础上，横向介绍该项技术在多个领域的应用，如“双向电泳技术”、“流式细胞术”等。

在内容上，系列图书主要有以下两个显著特点。一是强调先进性——除了系统介绍常用和经典实验技术以外，特别突出了当前该领域实验手段的新理论、

新技术、新发展，为国内专业人员起到借鉴和引导作用。二是强调可操作性——对于每一项实验技术，系统介绍其原理方法、设备仪器和实验过程，让读者明了实验的目的、方案设计以及具体步骤和结果处理，以期起到实验指南的作用。

本系列图书坚持质量为先，开拓国内和国际两个出版资源。一方面，约请国内相关领域兼具理论造诣和丰富实验室工作经验的专家学者编著；另一方面，时刻关注国际生命科学前沿领域和先进技术的进展，及时引进（翻译或影印）国外知名出版社的权威力作。

“生物实验室系列图书”的读者对象设定为国内从事生命科学及生物技术和相关领域（如医学、药学、农学）的专业研究人员，企业或公司的生产、研发、管理技术人员，以及高校相关专业的教师、研究生等。

我们殷切希望“生物实验室系列图书”的出版能够服务于我国生命科学的发展需要，同时热忱欢迎从事和关心生命科学的广大科技人员不仅对已出版图书提供宝贵意见和建议，也能对系列图书的后续题目设计贡献良策或推荐作者，以便我们能够集思广益，将这一系列图书沿着可持续发展的方向不断丰富品种，推陈出新。

谨向所有关心和热爱生命科学，为生命科学的发展孜孜以求的科学技术工作者致以崇高的敬意！

祝愿我国的科技事业如生命之树根深叶茂，欣欣向荣！

化学工业出版社

生物·医药出版分社

序

在 20 世纪，从发现 DNA 作为遗传物质的基本形式、揭示基因的化学和结构本质、建立遗传中心法则到完成人类基因组测序，人类在探索生命奥秘的领域中取得了瞩目的成绩。尽管应用于绝大多数生命形式的中心法则表明，遗传信息是从 DNA 到信使 RNA (mRNA)，再到蛋白质，但是对具有 30 亿密码的人类基因组的全部测序，并没有阐明数以万计基因的单向信息流是如何精确编程的。如果把人类基因组一维线性遗传密码的完全破译比作人类登上月球，那么在四维的生物学背景下以基因表达的形式解释遗传指令，例如在发育和疾病过程中，其复杂性和困难程度将是比人类从月球返回地球更富于挑战性、更令人生畏的任务。

尽管经典遗传学是研究由单基因编码蛋白的功能缺失引起的分子疾病的有效方法，但它在诸如癌症、乙型糖尿病及心脏病等多基因控制的表型研究中却乏善可陈。事实上，许多基因本身是信号分子，每一个都控制着一系列下游基因的表达。因此，分析差异基因表达或已故 Ruth Sager 提出的 RNA 遗传学，已经成为研究更为复杂的生物系统的一个最切实可行的策略。也许这方面最早成功的先例之一是 20 世纪 70 年代末期 p53 肿瘤抑制蛋白的发现，当用 DNA 肿瘤病毒侵染正常细胞时这种蛋白过量表达。后来，双向蛋白凝胶电泳的发展绘制了更为完整的胞内蛋白表达图谱。在 20 世纪 80 年代早期出现了一些针对 mRNA 表达的研究方法，例如差异筛选和消减杂交在基因鉴定方面比双向蛋白电泳更全面、更灵敏，并且能够提供更多的信息。Mark Davis 和他的同事用这种策略比较 T 细胞和 B 细胞 mRNA 表达的差异时，发现了 T 细胞受体，这为通过基因表达的分析来发现新基因提供了最完美的例子。T 细胞受体的成功发现为生物医学研究注入了新的活力，以基因表达分析为基础策略可用于各种生物学系统的研究。20 世纪 90 年代，人们发明了若干新的、更为复杂的分子生物学工具，在 mRNA 水平上全面分析基因表达。这些方法，包括差异显示 (DD)、基因表达的系列分析 (SAGE) 和 DNA 微阵列，已经应用于基因表达分析的大量研究中。在 Medline 中，DD、SAGE 和 DNA 微阵列的点击数已超过 6000！其后，这些技术的各种改进，以及集中于体内和体外各种水平的基因表达分析的新方法最近已经有报道。

这本命名为《基因表达分析手册》的书，由德国科学家 Stefan Lorkowski 博士以及爱尔兰医学博士 Paul Cullen 编写，Wiley-VCH 出版社出版，花费数年心血完成，代表了全世界 200 多位研究者的集体成就，他们中的许多人都是基