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研究方法与实验方案（原著第2版）

Mitochondrial DNA

Methods and Protocols (Second Edition)

Jeffrey A. Stuart

科学出版社

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导　读

线粒体是胞内细胞器，在细胞的生命活动过程中承担了重要的功能。除了作为细胞的能量工厂生产 ATP 外，线粒体广泛参与细胞内脂类、胆固醇、氨基酸和核苷酸的代谢，在细胞的信号转导、自噬和凋亡过程中也承担了重要的角色。近期研究显示线粒体还参与天然免疫。线粒体拥有自身遗传物质线粒体 DNA（mtDNA），编码 13 种氧化磷酸化呼吸链组成蛋白、22 种转运 RNA 和 2 种核糖体 RNA。虽然由线粒体自身基因组编码的蛋白仅占线粒体总蛋白种类的 1%左右，但 mtDNA 基因突变、复制、转录和翻译异常，往往会导致线粒体功能缺陷，进而表现为组织和器官功能异常，导致临床症状非常复杂的线粒体病。目前，线粒体生物学和线粒体病的研究在国际上是持续的热点。

得益于我们对线粒体知识的积累，同时更多地是研究技术方法的创新和改进，线粒体生物学和相关疾病研究近几十年来发展突飞猛进。瞄准这一发展态势和契机，Springer 旗下的 Humana 出版社 2002 年在其旗舰系列丛书《分子生物学研究方法》中及时出版了由 William C. Copeland 主编的《线粒体 DNA：研究方法与实验方案》一书，系统地介绍了 mtDNA 突变研究方法和控制 mtDNA 转录的关键蛋白的表达、纯化和功能鉴定。2009 年，该出版社又出版了由 Jeffrey A. Stuart 主编的《线粒体 DNA：研究方法与实验方案》第二版，以汇总和更新第一版出版后本领域研究的一些新方法和新进展，其中重点介绍了控制 mtDNA 复制、转录、翻译和呼吸链复合物组成蛋白的表达、纯化和功能鉴定方法。对于第一版中已有介绍的一些以检测 mtDNA 突变和 DNA 损伤为目的的研究方法，本版中作者重点介绍了不同于以前介绍的技术手段或改进的技术方法来达到同一研究目的。参与这两版章节撰写的绝大多数作者都是目前国际上该领域前沿和一线的研究人员，描述的研究方法也由他们首创或在前人基础上做出重大改进，他们分享的实验过程中需要注意的技巧和一些容易发生的错误对于初涉 mtDNA 领域的研究者来说尤为重要，可以帮助研究者在实验过程中避免常见的实验错误，尽快得到准确的分析结果。

诚然，作为一本特定介绍 mtDNA 研究方法和实验指南的工具书，其专业性相当强，阅读起来也比较晦涩难懂，尤其是对于没有先期接触一些 mtDNA 生物学知识的读者。另外，就是书中介绍的一些研究方法，如二维琼脂糖电泳识别 mtDNA 复制中间体，从实验体系的稳定建立到实验数据的解释，都需要结合具体的实验过程才能够更好地理解具体的实验要求和操作细节。同样，对于介绍的一些实验方法，如基于变性高效液相色谱（DHPLC）异源双链分析法测定 mtDNA 突变异质性、单细胞 mtDNA 突变分析等，在实验开展过程中需要配置一些特定的仪器设备。离开这些硬件支持，将不能够重复出本书作者介绍的实验。这些都是本书比较明显的缺点，也限制了它的读者群。

在第二版中，对于同一种蛋白的体外表达和功能鉴定，如线粒体单链 DNA 结合蛋白和线粒体 DNA 解旋酶，不同作者分别以独立章节介绍了他们采用的实验条件和流程。这些控制 mtDNA 复制和转录的相关重要蛋白的表达纯化和功能鉴定等生化分析在第一版中也有介绍，在内容上有些重复。不过，这对于我们在研究中如何尽快建立起稳定的实验体系，无疑提供了更多的选择和可供比较的资料。所以，对于国内研究者，如

果希望在自己的实验室建立起本书中描述的相关生化分析方法，一个建议就是将第一版和第二版中关于同一功能蛋白的相关实验流程对比阅读，同时结合实验室已有条件，选择出最适合开展的一种方法。

由于本书是按照《分子生物学研究方法》系列丛书的写作格式来编排，在每个章节开始部分都有关于该研究方法的目的意义或者是所研究功能蛋白的整体介绍，并引用了若干文献。这些介绍和文献可以给读者提供较为丰富的信息，通过阅读引用的原文献，可进一步加深理解和获取相关专业知识。读者在读本书之前，不妨阅读 Wiley-Liss 出版社出版的由 Immo E. Scheffler 教授编著的《线粒体》一书，来获取更多的关于线粒体生物学的基础知识。另外，学术出版社（Academic Press）的系列丛书《细胞生物学研究方法》中出版的由 Liza A. Pon 和 Eric A. Schon 教授主编的《线粒体》（第二版），也是一本很好的了解线粒体研究方法的参考书和工具书。

目前国内研究者关于 mtDNA 的研究多集中在序列变异分析方面，将 mtDNA 作为一个遗传标记来探讨群体的遗传多样度，评估家养动物品系和野生动物的遗传濒危程度，重建家养动物和人类民族群体的起源和迁移等事件。对于母系遗传病和衰老、肿瘤等组织中发现的 mtDNA 突变及其潜在功能也有一些研究，但整体水平有待进一步提高，尤其是关于 mtDNA 复制、转录、翻译、氧化呼吸链复合物功能鉴定、线粒体核质互作等研究需要特别加强。欣闻《线粒体 DNA：研究方法与实验方案》第二版将由科学出版社以导读版的形式引进国内出版，我觉得这对于提升国内 mtDNA 研究水平，培育更多的对 mtDNA 研究充满兴趣和激情的年轻学者无疑有着重要的作用。我期待有更多的年轻学者加入 mtDNA 研究俱乐部！

姚永刚
中国科学院昆明动物研究所
二零一一年十二月

前　言

从2002年第一版《线粒体DNA：研究方法与实验方案》发行至今，已有大量的mtDNA突变被发现与生物能量代谢异常、细胞死亡及疾病相关。同时，我们对mtDNA体细胞突变的生物学功能的了解也在逐步加深。这些进步在很大程度上得益于线粒体生物学和mtDNA研究方法的不断发展和改进。在第二版的《线粒体DNA：研究方法与实验方案》中，来自8个国家的mtDNA研究领域的专家分享了针对mtDNA各研究方向的详细的研究方法。

本书主要内容分为三部分。第一部分介绍了从mtDNA分子到氧化呼吸链复合物合成组装这一通路的相关研究方法。具体来说，这一部分阐述了mtDNA核状小体、mtDNA组装、复制、转录及呼吸链复合物合成的相关研究方法，其中包含了研究与线粒体病相关的mtDNA聚合酶γ突变的方法。第二部分主要关于线粒体活性氧簇（ROS）产生，以及mtDNA损伤和修复。其中包括用于研究和评估mtDNA损伤修复能力的专门的实验体系。这些实验方法的应用将有助于加深我们对mtDNA损伤和修复及mtDNA突变的生物学功能的了解。本书第三部分基于在衰老相关疾病中观察到的与生物能量代谢缺陷相关的mtDNA体细胞突变，介绍了mtDNA异质性突变鉴定和定量的新方法。

本书中除了介绍早期建立并沿用至今的完整详细的技术方案，还涵盖了一些新近发展的可能非常有助于我们了解mtDNA生物学特性的研究方法。在此书中，研究生、博士后和知名研究者都能够找到非常直观易懂的有用信息，并且能够将这些足够详细的研究方法在他们自己的实验室中应用起来。感谢为撰写此书付出辛勤汗水的作者，感谢你们的奉献及莫大的耐心，同时也感谢你们无私地与大家分享经验及详尽的研究方案。

Jeffrey A. Stuart

（姚永刚　译）

Preface

Since the publication of the first edition of *Mitochondrial DNA: Methods and Protocols* in 2002, the number of unique heritable mtDNA mutations recognized as being associated with bioenergetic dysfunction, cell death, and disease has grown apace. At the same time, our understanding of the basic biology of somatic mtDNA mutations has improved. These ongoing advancements are due largely to the continuous development and improvement of techniques and approaches for studying the biology of mitochondria and their DNA. In this second edition of *Mitochondrial DNA: Methods and Protocols*, specialists from eight countries share their expertise by providing detailed protocols for studying many aspects of mtDNA.

This volume is divided into three sections. The first contains protocols that can be used to study the transduction of information from mtDNA to functionally active respiratory complexes. Included in this section are protocols for investigating the nucleoid proteome, mtDNA packaging, replication, transcription, and respiratory complex synthesis. In this section, methods for studying polymerase gamma mutations associated with mitochondrial disorders are also provided. The second section focuses on mitochondrial reactive oxygen species (ROS) production, mtDNA damage, and its repair. Included are descriptions of unique experimental systems for manipulating mtDNA repair capacities and evaluating the outcome. The application of such methods will improve our understanding of the basic biology of mtDNA damage, repair, and mutation. Finally, in the third section, in recognition of the observation that debilitating somatic mtDNA mutations underlie some of the bioenergetic deficits observed in age-associated disease, exciting new approaches for identifying and quantifying heteroplasmic mtDNA mutations are presented.

This volume contains detailed descriptions both of established techniques that continue to be usefully applied, and of some very recently developed approaches that hold great potential to improve our understanding of mtDNA biology. As such, graduate students, postdoctoral fellows, and established investigators should all find herein useful information presented in a straightforward manner with sufficient detail to be replicated in their own laboratories. I thank all of the authors who contributed their expertise and detailed protocols to this volume for their hard work, dedication, and patience.

Jeffrey A. Stuart

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目　录

（姚永刚 译）

Contents

Part I

Mitochondrial DNA Replication, Transcription, Translation, and Respiratory Complex Assembly

Chapter 1

Biochemical Isolation of mtDNA Nucleoids from Animal Cells

Daniel F. Bogenhagen

Abstract

Mitochondrial DNA (mtDNA) in animal cells is organized into clusters of 5–7 genomes referred to as nucleoids. Contrary to the notion that mtDNA is largely free of bound proteins, these structures are nearly as rich in protein as nuclear chromatin. While the purification of intact, membrane-bound mitochondria is an established method, relatively few studies have attempted biochemical purification of mtDNA nucleoids. In this chapter, two alternative methods are presented for the purification of nucleoids. The first method yields the so-called native nucleoids, using conditions designed to preserve non-covalent protein–DNA and protein–protein interactions. The second method uses formaldehyde to crosslink proteins to mtDNA and exposes nucleoids to treatment with harsh detergents and high salt concentrations.

Key words: mtDNA, mitochondria, nucleoids, chromatin IP.

1. Introduction

The maintenance of mitochondria depends on the mitochondrial DNA (mtDNA) for synthesis of several protein components of the oxidative phosphorylation machinery. In mammals, 13 proteins are encoded in the mtDNA genome along with 12S and 16S rRNAs and a complete, albeit minimal, complement of 22 tRNAs. The 13 proteins synthesized on mitochondrial ribosomes are incorporated into respiratory complexes I, III, IV, and V along with approximately 67 nucleus-encoded subunits *(1)*. Cells typically maintain thousands of copies of mtDNA distributed among hundreds of organelles that exchange components through active cycles of fusion and fission *(2, 3)*. These mtDNA genomes are organized in nucleoids containing 2–10 genomes, as indicated in **Table 1.1** *(4)*.

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