

METHODS IN MOLECULAR BIOLOGY™

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Cell Cycle Synchronization

Methods and Protocols

Edited by

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Preface

What Kind of Cells Can Be Synchronized?

To study how cells progress through the cell cycle, cell cultures have to be brought to the same phase. The unique feature of this book is exactly this: to prepare synchronized cells representing different stages of the cell cycle. The book also shows the latest techniques for the enhanced study of regulatory mechanisms to understand cell cycle events. The synchronization methods presented in the book are based principally on two major strategies. The “arrest-and-release” approach involves different chemical treatments to block cells at certain stages of the cell cycle. The physical strategy contains physical methods to collect cells belonging to subpopulations of the cell cycle. The collection of synchronized cells from asynchronous bacterial, plant, protozoan, yeast, fish, and mammalian cell cultures consisting of individual cells is described by professionals of their respective field. Additional chapters include synchronization of transfected and embryonic cells.

Why Edit a Book on Synchronization?

This question can be easily answered by making a computer search typing in the words “cell synchronization.” It turns out that there are many scientists who are desperately looking for synchronization protocols. Researchers are interested in synchronizing mammalian, plant, yeast, fungal, and even bacterial cells but do not know how to do it. Enthusiastic students often try synchronization without experience and then recognize that it does not work. In such cases, the easiest way is to ask someone known for his/her expertise to send a protocol. As some of the synchronizing techniques are tricky, brief instructions usually would not help either. Alternatively, one can trace research papers that contain descriptions without extensive practical details but describe only rarely how problems encountered could be solved. This book aims to address such deficiencies. But the most important feature of this book is to supply detailed protocols providing first the theoretical background of the procedure and then step-by-step instructions on how to implement synchronization. Chapters of the book are written for those competent scientists who would like to do, but are not familiar with, synchronization. They should be able to carry out successfully the technique at the first attempt by following closely the detailed practical instructions of the protocols.

Major Sections of Chapters

Each protocol starts with an Abstract and consists of four major sections: Introduction, Materials, Methods, and Notes. Exceptions are only the first and last review chapters

that do not follow this format. The “Abstract” gives an overview of the synchronization technique(s). The “Introduction” contains a summary, a brief theoretical view of the procedure referring to the work of other authors, and outlines the major procedures of the protocol. The “Materials” section is the major part of the chapter listing the buffers, reagents, solutions, disposables, and equipments necessary to carry out the synchronization. Attention is called to special requirements such as storage conditions, stability, purity, toxicity of reagents, special treatment, or protection. The “Methods” section contains all relevant practical details and explains individual steps to be carried out normally by listing these steps in numerical order. The “Notes” section is the hallmark of the series of *Methods in Molecular Biology* as it is meant to indicate the sources of problems and how to identify and overcome them.

Brief Content of Chapters

The introductory chapter overviews synchronization methods. Chapters of physical fractionations include centrifugal elutriation (**Chapter 2**), cytofluorometric purification of diploid and tetraploid cancer cells (**Chapter 3**) and large-scale mitotic cell synchronization (**Chapter 4**), as well as applying serum deprivation (**Chapter 5**). Chemical blockades imply inhibitors of DNA replication (**Chapters 6 and 7**), butyrate (**Chapter 8**), nocodazole to arrest cells at the G₂/M border, and aphidicolin to synchronize cells at the G₁/S border and to monitor progression through S phase by pulse-labeling individual cultures with [³H]-thymidine (**Chapter 9**). Different ways have been used to synchronize HeLa cells in **Chapter 10**. The synchronization of unicellular organisms (*Bacillus subtilis*, yeast, protozoans) is described in **Chapters 11, 12, and 13**. The synchronization of mammalian and plant cells is studied in **Chapters 14 and 15**, respectively. A protocol for the synchronization for the purposes of nuclear transfer is given in **Chapter 16**. Hematopoietic stem cells improve the engraftment in transplantation (**Chapter 17**). Flow cytometry developments in clinical studies are described in **Chapter 18**. Finally cell cycle control is discussed (**Chapter 19**).

Which Is the Best Synchronization Protocol?

It is neither the intention of this book to make a judgment as to which synchronizing procedure is the best or to set a “gold standard” against which other methods should be measured. Debates on synchronization methodologies can be found in opinion papers referred to in **Chapter 1** (14–18). A simple method for obtaining synchrony in all types of cells, that would last through several cycles and with minimal overall metabolic perturbations, does not exist. Thus scientists interested in synchronization after reading the chapters of interest can decide for themselves which technique would be appropriate for adaptation.

The Potential Audience of This Book

First of all, those students and scientists who are looking for synchronization protocols will be interested. The main target audience includes

- libraries of universities and biological research institutions;
- researchers interested in general science, pharmacy, medicine and public health, computer science, and the life sciences;
- specialists and professionals in cell biology, genetics, molecular biology, biochemistry, and pharmacology;
- biologists, molecular biologists, biotechnologists, geneticists, immunologists, medical students, PhD students, and postdoctoral fellows who are expected to be the primary users of the synchronizing techniques and protocols;
- pharma companies and factories.

Gaspar Banfalvi

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