# METHODS IN MOLECULAR BIOLOGY

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# Cell Cycle Synchronization

## Methods and Protocols

### Second Edition

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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.

## What Motivated the Second Edition of *Cell Cycle Synchronization*?

The interest in the first edition of *Cell Cycle Synchronization* is indicated by the 49,074 chapter downloads between 2012 and February 2016. There are always scientists who are desperately looking for synchronization protocols. Researchers are interested in synchronizing mammalian, plant, yeast, fungal, and even bacterial cells, but often do not know how to do it. Enthusiastic students 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 updated book is to give detailed protocols providing first the theoretical background of the procedure then step-by-step instructions on how to implement synchronization and finally how to avoid pitfalls referred to in the Notes. 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. If your attempt would fail, you can still contact and ask preferentially the first or last author about the technical details of the chapter you are interested in.

## 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 equipment 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 "Materials" 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 of healthy and apoptotic cells, and nuclei of mammalian cells (Chapter 2), image cytofluorometry for the quantification of ploidy and stress of cancer cells (Chapter 3), and large-scale mitotic cell synchronization (Chapter 4). Chemical blockades imply intervention on the spindle assembly checkpoint (Chapter 5), synchronization by serum deprivation (Chapters 6 and 10), DNA replication stress inhibitors (Chapters 7), chromosome formation during fertilization in eggs (Chapter 8), synchronization with butyrate (Chapters 8 and 10), nocodazole to arrest cells at the $G_2$/M border, and aphidicolin to synchronize cells at the $G_1$/S border and to monitor progression through S phase by pulse-labeling individual cultures with [$^3$H]-thymidine (Chapter 11). Different ways have been used to synchronize HeLa cells in Chapter 12. The synchronization of unicellular organisms (*Bacillus subtilis*, yeast, protozoans) is described in Chapters 13–15. The synchronization of maturation of porcine oocytes is described in Chapter 16. Plant cell synchronization is dealt with in Chapter 17. A protocol for the synchronization for the purposes of nuclear transfer is given in Chapter 18. Hematopoietic stem cells improve the engraftment in transplantation (Chapter 19). Flow cytometry developments and perspectives in clinical studies are described in Chapter 20. Finally, cell cycle control is discussed in Chapter 21.

## Which Is the Best Synchronization Protocol?

It was neither the intention of the first edition nor the second edition of this book to make judgement as to which synchronizing procedure would be the best or to set a "gold standard" against which other methods should be measured. Debates on cell-cell 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, which 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 by themselves which technique would be appropriate for adaptation.

## The Potential Audience of This Book

Primarily those students and scientists were interested in the first edition who were looking for synchronization protocols. The main target audience includes the following:

- libraries of universities and biological research institutions,
- researchers interested in general science, pharmacy, medicine and public health, computer science, and the life sciences;
- specialist and professionals in cell biology, genetics, molecular biology, biochemistry, 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.

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