

# METHODS IN MOLECULAR BIOLOGY™

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# **Nucleic Acid Detection**

## **Methods and Protocols**

Edited by

**Dmitry M. Kolpashchikov and Yulia V. Gerasimova**

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 **Humana Press**

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ISSN 1064-3745                      ISSN 1940-6029 (electronic)  
ISBN 978-1-62703-534-7            ISBN 978-1-62703-535-4 (eBook)  
DOI 10.1007/978-1-62703-535-4  
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013943589

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Printed on acid-free paper

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## Preface

Nucleic acid detection has an outstanding potential in molecular diagnostics of cancer, infectious diseases, and genetic disorders. It is undoubtedly successful in environmental monitoring, food control, genetic linkage analysis, as well as in forensic casework. Some of the early sequence-specific detection techniques were introduced in the early 1960s and have been evolving and diversifying since then. Among most useful modern technologies the following are of general importance: gel and capillary electrophoresis (CE), polymerase chain reaction (PCR), quantitative real-time PCR (qPCR), DNA microarrays, fluorescent in situ hybridization (FISH), and Southern blot. This volume is a collection of techniques and emerging approaches for the detection of both DNA and RNA. The presented protocols reflect some of the trends in improving nucleic acid detection methods.

Chapter 1 is relevant to forensic DNA analysis and describes an efficient strategy for the recovery of trace amounts of DNA from touched objects followed by its amplification for the analysis of short tandem repeats. Chapter 2 describes a technique for tissue sample preparation that keeps the morphology intact, thus further RNA analysis can reveal the cancerous components from non-cancerous.

Chapters 3 and 4 describe new qPCR chemistries. One common advantage of the methods is the possibility of using the same fluorescent reporter for many targeted sequences, which may significantly reduce the cost of qPCR reagents. Chapters 5 and 6 describe other qPCR compatible cost-efficient fluorescent probes that have an additional advantage of improved selectivity and specificity. Overall, there is a trend of using *universal* fluorescent reporters adaptable for the detection of any DNA or RNA target in real-time formats, which promises to make probe-based qPCR more affordable in the near future.

As alternatives to PCR, isothermal DNA amplification techniques are becoming popular. They have the advantage of eliminating the need for PCR thermal cycler thus representing less-demanding and affordable alternatives to PCR in point-of-care (POC) diagnostics. This volume includes examples of helicase-dependent amplification (Chapter 7) and loop-mediated isothermal amplification (LAMP) (Chapters 8–10).

An alternative to DNA amplification is signal amplification approach, which has received significant attention in the last decade. These types of assays do not amplify the amount of target DNA. Instead, the presence of an analyte activates enzymes that amplify the signal by multiple processing of a (fluorogenic) substrate. Invader assay and 5' fluorogenic exonuclease assay (TaqMan) are examples of such approaches. Yet another signal amplification technique is detailed in Chapter 11 of this book.

Most common methods for nucleic acid detection rely on fluorescent outputs. However, the demand of POC diagnostics calls for the assays with visually recognized signals. Such assays do not require any instrumentation and minimize the processing time and the user expertise required. Techniques based on peroxidase-like DNA enzyme (Chapters 12 and 13) and gold nanoparticles (Chapter 14) are included in this volume. Other alternatives to fluorescence are electrochemiluminescence (Chapter 15), interferometric reflectance imaging (Chapter 16), and electrochemical detection using graphene oxide (Chapter 17).

FISH has been employed for the chromosome analysis since the early 1980s but still undergoes improvements (Chapter 19) and faces new applications (Chapter 2). A FISH-related technology uses short peptide nucleic acid (PNA) strands to unwind a local dsDNA fragment followed by sequence-specific analysis of the opened fragment (Chapter 18). This very promising approach has the advantages of sequence specificity, low detection limits, and mild hybridization conditions.

In recent years significant attention was devoted to the detection of micro RNAs (miRNAs) as they are considered to be important cancer biomarkers (Chapters 2, 20–22). Another extensively explored application is RNA imaging in live cells. This field is driven both by the great success of green fluorescent protein-based protein imaging and growing understanding of the great diversity and importance of intracellular RNA. Chapters 23 and 24 describe new hybridization probes that might be useful for the purposes of intracellular RNA monitoring.

Besides the traditional issues of detection limits, selectivity, and reliability, the one common trend in the new nucleic acid detection methods is to make them suitable for POC diagnostics, which means, reduced assay time, cost efficiency, and simple and straightforward formats. The other trend is detection of micro RNA and RNA in living cells. While one volume cannot accommodate all the existing developments in the field of nucleic acid detection we hope that the presented methods will be useful for those who are interested in DNA or RNA analysis.

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