METHODS IN MOLECULAR BIOLOGY

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In Situ Hybridization Protocols

Fourth Edition

Edited by

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Humana Press

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Molecular Histology
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ISSN 1064-3745 ISSN 1940-6029 (electronic)
ISBN 978-1-4939-1458-6 ISBN 978-1-4939-1459-3 (eBook)
DOI 10.1007/978-1-4939-1459-3
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2014944535

Printed on acid-free paper

Humana Press is a brand of Springer
Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Ribonucleic acid (RNA) molecules are fragile single-stranded transcripts of genetic DNA, generally considered as the coding messenger needed to produce protein from the DNA. These RNA messengers (mRNA) have together with transfer RNA (tRNA) and ribosomal RNA (rRNA) historically been considered the essentials in RNA biology, driving the translation of RNA into protein. Today it is known that only a minor percentage of all transcribed RNA is translated into protein and that several noncoding RNA species exist and may have vital functions. These RNA species include long noncoding (lnc) RNA, circular RNA, vault RNA, microRNA (miRNA) among others. Whereas the mRNAs constitute the basis of protein synthesis, a general function cannot be attributed to the novel noncoding RNA species other than they contribute to regulation of molecular expression and interaction. Visualization of these novel noncoding RNA species in cells and tissues is an important tool to help understand their roles in cell and tissue biology. Therefore, RNA in situ hybridization, the visualization of RNA molecules in cells and tissues, has become an increasingly important technology within the last decade. This fourth edition of "In Situ Hybridization Protocols" contains 21 protocols that utilize the in situ hybridization technology to document or take advantage of the visualization of specific RNA molecules.

In parallel with the discovery and characterization of novel RNA species, novel tools to detect the RNA species in situ have also been invented and developed into commercial probes and kits that are presented in this edition. For example, using branched DNA technology, S. Zolotukhin shows an application of the Panomix/Affymetrix' kit Quantigene®, and Ma et al. report on applications of ACD's RNAscope® detection kits. Use of the multiple oligo approach in the Stellaris® probe design from Biosearch Technologies is shown by Coassin et al. to simultaneously detect mRNA and lncRNA species in cultured cells. Locked nucleic acid (LNA)-based probes are the general probe technology used for miRNA detection in tissues and cells (Goossens et al., Nielsen et al., Renwick et al., LF. Sempere, A. Silahtaroglu, Wu et al., and Turnock-Jones and Le Quesne), but have also been applied for mRNA detection by Darnell and Antin, and for fungal rRNA detection by KT. Montone. Furthermore, the peptide nucleic acid (PNA) probe design is presented for detection of bacterial rRNA in clinical samples by Fazli et al. Traditional riboprobes generated by in vitro transcription are utilized in several protocols: Dakou et al., Fuentes and Fernandez, Rybak-Wolf and Solana, Stylianopoulou et al., Thisse and Thisse, and Usher et al.

Probe detecting reagents are important for signal amplification and visualization of the RNA species and can be divided into chromogenic dyes and fluorescent dyes. Whereas applications using chromogen staining are advantageous for single-molecule detection, detection with fluorescent dyes also allows multiplex staining as exemplified in the protocols presented by LF. Sempere and Renwick et al.

In situ hybridization-based detection of RNA is possible in a variety of tissue species, and includes tissue sections from archived human pathological tissue samples, processed by formalin fixation and paraffin embedding, so-called FFPE samples, snap frozen tissue samples, and whole mount samples. Development of in situ hybridization technologies for FFPE samples is essential for future implementation of RNA in situ hybridization in

a clinical setting. Applications on clinical FFPE sections are exemplified by studies in tissue microarrays (TMA) by Turnock-Jones and Le Quesne, multiplex staining applications by LF. Sempere, Ma et al., and Renwick et al., as well as studies on fungi and bacterial rRNA by KT. Montone and Fazli et al., *respectively*. In situ hybridization analysis of experimental FFPE samples with mouse tissue or cultured cells is presented by Usher et al. Applications on frozen tissue sections are presented by A. Silahtaroglu on mouse samples and Nielsen et al. on human samples. In situ hybridization in whole mount samples is restricted to small size specimens, but allows investigation of early stages of development and provides a excellent spatial localization of RNA expression as presented in mouse embryos by Dakou et al., zebrafish oocytes to larvae by Fuentes and Fernandez, zebrafish embryos by Thisse and Thisse, bovine blastocysts by Goossens et al., and flatworms/planarian by Rybak-Wolf and Solana.

The in situ hybridization technology has a natural limitation in sensitivity. The branched DNA probe technology, presented by Ma et al. and S. Zolotukhin, and Stellaris® probe technology presented by Coassin et al., indicate single-molecule detection. Important tissue fixation steps are required to retain intact RNA inside the tissue samples, and here Stylianopoulou et al. present advantages with a zinc-based fixative. Using the in situ hybridization technology in other branches of molecular cell biology is exemplified by two contributions. First, Own and Patel used RNA in situ hybridization to localize areas of interest for DNA methylation studies. And second, Wu et al. show recent advances in the application of LNA probes and their use to identify cells containing specific RNA targets in flow cytometry.

In situ hybridization protocols are generally considered extensive and complicated with several hands on steps. One approach to fully automation of the miRNA in situ hybridization protocol is presented by LF Sempere. All contributors to this book have included a list of notes that reveal practical experiences gained by the authors, and will be of precious help for beginners as well as informative for the experienced researchers who wish to learn from others specialized in the field.

It is evident that this fourth edition of "In Situ Hybridization Protocols" reflects that the area of RNA in situ hybridization has broadened extensively over the last decade, thanks to the invention and development of several new probes and detection technologies. The continued discovery of new RNA species and uncovering of their cellular functions is an indication of a high need for continued exploration and improvement of probe technologies in this field.

Finally, I would like to thank all authors who kindly contributed with the thorough protocols and provided extensive technical details and recommendations for the users.

Hørsholm, Denmark ***Boye Schnack Nielsen***

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