

Methods in Enzymology

Volume 318

RNA-Ligand Interactions

Part B
Molecular Biology Methods

EDITED BY

Daniel W. Celander

LOYOLA UNIVERSITY
CHICAGO, ILLINOIS

John N. Abelson

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA



ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

Table of Contents

| | |
|-------------------------------------|------|
| CONTRIBUTORS TO VOLUME 318. | ix |
| PREFACE. | xiii |
| VOLUMES IN SERIES | xv |

Section I. Solution Probe Methods

| | | |
|---|--|----|
| 1. Probing RNA Structure and RNA-Ligand Complexes with Chemical Probes | CHRISTINE BRUNEL AND PASCALE ROMBY | 3 |
| 2. Methylene Blue-Mediated Cross-Linking of Proteins to Double-Stranded RNA | ZHI-REN LIU, BRUNO SARGUEIL, AND CHRISTOPHER W. J. SMITH | 22 |
| 3. Structure and Distance Determination in RNA with Copper Phenanthroline Probing | THOMAS HERMANN AND HERMANN HEUMANN | 33 |
| 4. Applications of Uranyl Cleavage Mapping of RNA Structure | NIELS ERIK MØLLEGAARD AND PETER E. NIELSEN | 43 |
| 5. Identification of Messenger RNAs That Contain Inosine | DANIEL P. MORSE | 48 |

Section II. Tethered-Probe Methodologies

A. Photochemical Reagents

| | | |
|---|--|-----|
| 6. Site-Specific 4-Thiouridine Incorporation into RNA Molecules | YI-TAO YU | 71 |
| 7. Nucleoprotein Photo-Cross-Linking Using Halopyrimidine-Substituted RNAs | KRISTEN M. MEISENHEIMER, PONCHO L. MEISENHEIMER, AND TAD H. KOCH | 88 |
| 8. Structure Determination by Directed Photo-Cross-Linking in Large RNA Molecules with Site-Specific Psoralen | DMITRI MUNDIS AND PAUL WOLLENZIEN | 104 |
| 9. Photolabile Derivatives of Oligonucleotides as Probes of Ribosomal Structure | BARRY S. COOPERMAN, REBECCA W. ALEXANDER, YURI BUKHTIYAROV, SERGUEI N. VLADIMIROV, ZHANNA DRUZINA, RUO WANG, AND NORA ZUÑO | 118 |

- | | | |
|--|---|-----|
| 10. Photoaffinity Cross-Linking and RNA Structure Analysis | BRIAN C. THOMAS, ALEXEI V. KAZANTSEV, JIUNN-LIANG CHEN, AND NORMAN R. PACE | 136 |
|--|---|-----|

B. Chemical Reagents

- | | | |
|---|--|-----|
| 11. Cleavage of RNA with Synthetic Ribonuclease Mimics | RICHARD GIEGÉ, BRICE FELDEN, MARINA A. ZENKOVA, VLADIMIR N. SIL'NIKOV, AND VALENTIN V. VLASSOV | 147 |
| 12. Site-Specific Sulfhydryl Groups for Study of RNA Conformation via Disulfide Cross-Linking | SNORRI TH. SIGURDSSON | 165 |
| 13. Directed Hydroxyl Radical Probing Using Iron(II) Tethered to RNA | SIMPSON JOSEPH AND HARRY F. NOLLER | 175 |

Section III. *In Vitro* Affinity Selection Methodologies

- | | | |
|--|--|-----|
| 14. <i>In Vitro</i> Selection of RNA Aptamers | KRISTIN A. MARSHALL AND ANDREW D. ELLINGTON | 193 |
| 15. <i>In Vitro</i> Selection and Characterization of RNAs with High Affinity to Antibiotics | SCOT T. WALLACE AND RENÉE SCHROEDER | 214 |
| 16. Selection for RNA:Peptide Recognition through Sulfur Alkylation Chemistry | MATT WECKER AND DREW SMITH | 229 |
| 17. <i>In Vitro</i> Selection of RNA Substrates for Ribonuclease P and Its Catalytic RNA | FENYONG LIU, JUN WANG, AND PHONG TRANG | 238 |
| 18. RNA-Protein Interactions in Ribosomes: <i>In Vitro</i> Selection from Randomly Fragmented rRNA | ULRICH STELZL, CHRISTIAN M. T. SPAHN, AND KNUD H. NIERHAUS | 251 |
| 19. Optimized Synthesis of RNA-Protein Fusions for <i>In Vitro</i> Protein Selection | RIHE LIU, JEFFREY E. BARRICK, JACK W. SZOSTAK, AND RICHARD W. ROBERTS | 268 |

Section IV. Genetic Methodologies for Detecting RNA-Protein Interactions

A. Bacterial Systems

- | | | |
|---|---|-----|
| 20. Screening RNA-Binding Libraries by Transcriptional Antitermination in Bacteria | HADAS PELED-ZEHAVI, COLIN A. SMITH, KAZUO HARADA, AND ALAN D. FRANKEL | 297 |
| 21. Rapid Genetic Analysis of RNA-Protein Interactions by Translational Repression in <i>Escherichia coli</i> | CHAITANYA JAIN AND JOEL G. BELASCO | 309 |
| 22. RNA Challenge Phages as Genetic Tools for Study of RNA-Ligand Interactions | DANIEL W. CELANDER, KRISTINE A. BENNETT, DERRICK E. FOUTS, ERICA A. STEITZ, AND HEATHER L. TRUE | 332 |

B. Eukaryotic Systems

- | | | |
|---|---|-----|
| 23. Screening RNA-Binding Libraries Using Tat-Fusion System in Mammalian Cells | STEPHEN G. LANDT, RUOYING TAN, AND ALAN D. FRANKEL | 350 |
| 24. Frameshifting Assay to Characterize RNA-Protein Interactions in Eukaryotic Cells | HEIKE KOLLMUS AND HANSJÖRG HAUSER | 363 |
| 25. Translational Repression Assay Procedure: A Method to Study RNA-Protein Interactions in Yeast | EFROSYNI PARASKEVA AND MATTHIAS W. HENTZE | 374 |
| 26. Analysis of Low-Abundance Ribonucleoprotein Particles from Yeast by Affinity Chromatography and Mass Spectrometry Microsequencing | SCOTT W. STEVENS | 385 |
| 27. Yeast Three-Hybrid System to Detect and Analyze RNA-Protein Interactions | BEILIN ZHANG, BRIAN KRAEMER, DHRUBA SENGUPTA, STANLEY FIELD, AND MARVIN WICKENS | 399 |

Section V. Protein Engineering Methodologies Useful for RNA-Protein Interaction Studies

- | | | |
|--|--|-----|
| 28. Using Peptides as Models of RNA-Protein Interactions | COLIN A. SMITH, LILY CHEN, AND ALAN D. FRANKEL | 423 |
|--|--|-----|

| | | |
|---|---|-----|
| 29. Analysis of RNA-Protein Cross-Link Sites by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry and N-Terminal Micro- sequencing | BERND THIEDE AND BRIGITTE WITTMANN-LIEBOLD | 438 |
| 30. <i>In Vitro</i> Reconstitution of 30S Ribosomal Subunits Using Complete Set of Recombinant Proteins | GLORIA M. CULVER AND HARRY F. NOLLER | 446 |
| 31. Directed Hydroxyl Radical Probing of RNA from Iron(II) Tethered to Proteins in Ribonucleopro- tein Complexes | GLORIA M. CULVER AND HARRY F. NOLLER | 461 |

Section VI. Cell Biology Methods

| | | |
|--|---|-----|
| 32. Use of Dimethyl Sulfate to Probe RNA Structure <i>in Vivo</i> | SANDRA E. WELLS, JOHN M. X. HUGHES, A. HALLER IGEL, AND MANUEL ARES, JR. | 479 |
| 33. Sensitive and High-Resolution Detection of RNA <i>in Situ</i> | PASCAL CHARTRAND, EDOUARD BERTRAND, ROBERT H. SINGER, AND ROY M. LONG | 493 |
| AUTHOR INDEX | | 507 |
| SUBJECT INDEX | | 525 |