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## Preface

I hesitated a bit when I received an email from the Series Editor, John Walker, inviting me to consider producing an updated version of the *RNA–Protein Interaction Protocols* that I edited in 2008. I was pleased to know the volume was a success—at least in his opinion, but it was a long process and I might have gotten some authors frustrated because of my slow pace during editing. I finally decided to take on the task for I saw protocols for studying RNA–protein interaction are still being refined or developed anew, and the number of investigators engaging in RNA research continues to increase—many of them were in diverse fields and would benefit from detailed protocols.

I solicited protocols from authors who either developed or were very experienced with the method; I am very grateful for their willingness to contribute to this collection. By way of introduction, the first chapter uses two closely related RNA-binding proteins as examples to illustrate how various biochemical methods were applied to study the interactions between protein and RNA. Nineteen protocol chapters then follow, which can be categorized roughly in four sections.

The first section, from Chapters 2 to 5, describes ways to purify RNA–protein complexes assembled in cells or in isolated cellular extracts. The common strategy is to tag either the RNA with a biotin residue or the protein with the MS2 or the FLAG moiety so that the RNA–protein complexes can be readily purified by affinity.

The second section, from Chapters 6 to 9, describes methods for measuring various biochemical activities of RNA-interacting proteins or ribonucleoproteins. Protocols to assay the loading of small RNA onto argonaute protein, the role of ubiquitination in the assembly and disassembly of RNA–protein complexes, the kinetics of RNA methylation, and the reconstitution of small nucleolar RNPs are represented. The protocols contain specifics for individual experiments, yet they are written with a broad application in mind and are applicable to related studies.

The third section, from Chapters 10 to 14, features biochemical methods for measuring direct RNA–protein contact. Crosslinking protein and RNA in situ using chemicals followed by deep sequencing or proteomic detection generates data with high specificity and resolution. Together with Northwestern and polysomal profiling methods, these chapters may be viewed as the bread and butter of a typical RNA–protein methodology book.

The fourth section, from Chapters 15 to 20, collects various new or innovative methods pertinent to the subject. Cellular incorporation of a modified amino acid to a specified protein residue is adopted to pinpoint protein–protein interaction within an RNA–protein complex. A cell-based SELEX method is sophisticatedly expanded to isolate novel RNA molecules that can distinguish cell types. Elegant bioinformatic methods are developed to predict RNA–protein interactions and to design/manipulate RNA-binding proteins. Clever and effective reporters are constructed to assay spliceosome-mediated RNA splicing specificity or changes inside cells.

It would bring satisfaction if this book, in conjunction with the two previous editions of the *RNA–Protein Interaction Protocols* in the *Methods in Molecular Biology* series, provides a set of useful protocols, basic or advanced, to even a small number of researchers working with RNA and RNA-interacting proteins.

I would like to thank all the authors for their excellent contributions to this book, John Walker for his guidance, and the Springer editorial staff for their assistance. I am indebted to my students and colleagues who helped with this work and my wife for unconditional support.

*Duarte, CA*  
*On Thanksgiving of 2015*

*Ren-Jang Lin*



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