
Preface

Protein–protein interactions (PPIs) are strongly predictive of functional relationships among proteins in virtually all processes that take place in the living cell. Therefore, the comprehensive exploration of interactome networks is one of the major goals in systems biology. The development of “interactomics” as a field is largely driven by the development of innovative technologies and strategies for efficient screening, scoring, and validation of PPIs. The aim of this book is to provide a compendium of state-of-the-art protocols for the investigation of binary PPIs with the classical yeast two-hybrid (Y2H) approach, Y2H variants, and other *in vivo* methods for PPI mapping. Given the broad range of methodologies currently available, biochemical approaches like proteome-wide co-immunoprecipitation, and other *in vitro* and *in vivo* methodologies are not to be considered here. It needs to be emphasized, however, that alternative methods are very important for the complementation and validation of Y2H screens.

The book is structured into two sections. The first gives a survey of protocols that are currently employed for Y2H high-throughput screens by different expert labs in the field. Rather than detailing the principles of screening, which have been described previously, the focus is on different implementations of Y2H interactome mapping. First, two articles by Peter Uetz review the most important developments and applications of Y2H high-throughput screening. Then, Russ Finley, Ulrich Stelzl, Manfred Koegl, and coauthors describe their automated screening procedures in detail. A view on interactome research in pathogenic organisms is provided by Vincent Lotteau and Lionel Tafforeau (viral interactomes), and Douglas LaCount (interactomes of malaria parasites). Xiaofeng Xin and Thierry Mieg complement experimental protocols with their recently developed strategy of smart-pooling by shifted transversal design. Two more articles deal with bioinformatics for the analysis of Y2H data sets. Russ Finley and team discuss confidence scoring, whereas Gautam Chaurasia and Matthias Futschik describe the design of a database for high-throughput Y2H data (UniHI, Max Delbrueck Centrum, Berlin). John Reece-Hoyes and Albertha Walhout present a high-throughput yeast one-hybrid variant for the identification of proteins that bind-specific DNA segments. Finally, contributors from the lab of Young Chul Lee introduce their “one- plus two-hybrid system” for the efficient identification of PPIs altered by missense mutations.

The second part of the book considers innovative PPI detection methods that have the potential to emerge as alternative high-throughput methodologies. An important future role can be expected for systems that rely on the functional reconstitution (complementation) of reporter proteins by fused bait and prey proteins. A chapter on the split-ubiquitin-based system to screen for membrane protein interactions is provided by Igor Stagljar, whereas Mandana Rezwan and Daniel Auerbach of Dualsystems Biotech AG describe an approach to screen for interactors using the reconstitution of a split-TRP1 protein. For future human interactome studies, procedures that can reconstitute PPIs directly in mammalian cells could provide a better physiological context compared to yeast. A mammalian two-hybrid system based on the tetracycline-repressor system is presented by Kathryn Moncivais and Zhiwen

Zhang. A different principle in mammalian cells is used by Heinrich Leonhardt and team in their fluorescent two-hybrid approach, where bait and prey proteins are recruited to specific chromosomal locations. Perhaps the most advanced strategy for binary PPI mapping in mammalian cell culture is the mammalian protein–protein interaction trap (MAPPIT), developed by Jan Tavernier and his group. It is based on complementation of a cytokine receptor complex operating in mammalian cells. In the high-throughput ArrayMAPPIT application, prey proteins are arrayed in high-density microtiter plates to screen for interaction partners using reverse transfection into a bait-expressing cell pool. A variation of MAPPIT can be used to test substances that disrupt PPIs. Finally, Moritz Rossner provides a protocol for the use of uniquely expressed oligonucleotide tags (EXTs) that integrate complementation assays based on TEV protease and transcription factor activity profiling. Together, the protocols supply researchers with a comprehensive toolbox for the identification of biologically relevant protein interactions.

We are very grateful to all contributing authors for their great commitment to this project. We would like to express special gratitude to Dr. John M. Walker for his guidance and continuous support during the preparation of the manuscript.

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<http://www.springer.com/978-1-61779-454-4>

Two Hybrid Technologies

Methods and Protocols

Suter, B.; Wanker, E.E. (Eds.)

2012, XI, 329 p., Hardcover

ISBN: 978-1-61779-454-4

A product of Humana Press