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VOLUME 36

Yeast Gene Analysis

Second Edition

Edited by

Ian Stansfield & Michael J.R. Stark

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Cover image: **Yeast colony sectoring; a *Saccharomyces cerevisiae (ade2ade3)* strain transformed with a plasmid carrying a wild type copy of the *ADE3* gene forms colonies on agar plates that exhibit red/white sectoring due to random loss of the plasmid (see Chapter 15 'Smart Genetic Screens')**
I. Stansfield, University of Aberdeen

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Preface

The first edition of this volume was published in 1998, just over 2 years following completion of the *Saccharomyces cerevisiae* genome sequence and at the start of a period of concerted effort towards genome-wide functional analysis. With the genome sequencing complete, an integrated series of functional genomics approaches was needed, with the ultimate aim of assigning a role and biochemical function to each of the approximately 6000 proteins encoded by the genome.

The first edition of *Yeast Gene Analysis* (Brown and Tuite, 1998) was a very successful compendium of the current functional analysis methodologies at that time. However, since then a number of factors have combined to necessitate the update represented by this second edition. Firstly, great strides have been made through the introduction of many new, high-throughput and genome-wide approaches to yeast gene analysis that were either in their infancy or not even in existence when the first edition was published, such as microarray-based transcriptional profiling, high-throughput two-hybrid techniques, synthetic genetic array analysis and the development of protein microarrays. Secondly, a plethora of new molecular genetic and cell biology methodologies have been developed and applied to yeast functional genomics, including the use of TAP (tandem affinity purification)-tagging and improved mass-spectrometry analysis to study protein complexes and fluorescence resonance energy transfer (FRET), to name just two. Finally, many of the more established techniques applied to yeast functional analysis have been extensively developed, improved or re-evaluated over the past decade. The publication of this second edition therefore represents an ideal opportunity to assemble critical appraisals of these advances, together with experimental details as appropriate.

How has our understanding of the genome changed, and how much progress in functional analysis has been made, since publication of the first edition? The inventory of yeast genes has fluctuated a little, both through the elimination of falsely identified ORFs using comparative genomics (Cliften *et al.*, 2003; Kellis *et al.*, 2003; – see Chapter 22, this volume), and through new additions following the analysis of small ORFs (Kastenmayer *et al.*, 2006) and tiling array approaches (David *et al.*, 2006). However, excluding those ORFs still annotated as "dubious" the current size of the ORF set has not significantly changed. That over 800 potential genes remain within the "dubious" category underlines that there is still work to be done before we have a definitive gene set. The editors of the first edition of this book (Mick Tuite and Alistair Brown) included a table taken from Goffeau *et al.* (1996) summarising our genome-wide knowledge of yeast gene function at that time. This indicated 4343 ORFs that could be 'verified' because either they encoded known proteins (2611 ORFs) or showed similarity to other known or unknown proteins (1732 ORFs). Since at the time of

writing the *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org/) lists 4425 verified ORFs, how much progress have we really made since then? Although these statistics highlight a significant group of genes about which there is still little or no information available, these simplistic comparisons fortunately do not reveal the extent to which functional information on the *S. cerevisiae* gene set has been tremendously expanded by both high-throughput and smaller scale, focused studies over the past decade. For many of the characterised ORFs, we now know the localisation of the gene product, the likely interaction partners, loss of function phenotype, genetic interactions and how transcription of the gene varies under a wide range of conditions, or at least a useful subset of such information. Using SGD data (August 2006), there are 4160 genes assigned gene ontology (GO) term for molecular function. Even more ORFs have been assigned a biological process GO term (4824). However, despite this progress there are still 2112 ORFs with an unknown molecular function. Moreover, for some ORFs, even when a GO molecular function term has been assigned our knowledge regarding the precise function is sketchy to say the least. Functional analysis in yeast has made significant advances, but there is clearly still plenty to do.

Perhaps the best way to summarise the current state of play is to say that we now know a little about a lot of genes and a lot about a few genes. The experience of the last 8 years' efforts in yeast functional genomics now allows us to appraise the scale of the task of reversing this situation so that we can know a great deal about many (and ultimately, all) yeast genes. What has become clear is the value of high-throughput, genome-wide methodologies, both to provide an overall framework in which to carry out functional analysis and to ask specific questions on a genome-wide scale. When investigators identify a new gene in a focused study, the information on its localisation, transcriptional regulation and protein–protein interactions is frequently now already known, allowing rapid progress to be made in working outwards from a core process, gene set or protein complex towards identifying new gene products interacting with their system. Such approaches represent efficient ways to define an initial list of all the cellular components associated with a given gene, protein, process or environmental response. The new edition of *Yeast Gene Analysis* therefore brings together contributions covering both the high-throughput methodologies and those more suited to a gene-by-gene approach, representing an excellent primer for those adopting either style of research.

While the techniques covered by this new edition have obviously been expanded and revised, the new version is now even more focused on the budding yeast *S. cerevisiae*. Although since the first edition the genomes of both *Schizosaccharomyces pombe* and *Candida albicans* have been completed (Wood *et al.*, 2002; Jones *et al.*, 2004), each of these organisms is now associated with its own burgeoning suite of species-specific molecular genetic

methods for the analysis of gene function and as a result, *C. albicans* and *S. pombe* functional analysis could now each easily merit the entire coverage of a volume in their own right. We therefore made a conscious decision to restrict coverage in the second edition almost entirely to *S. cerevisiae*, especially given the expanded range of budding yeast functional genomics approaches. However, we have retained the format of the original volume, each chapter providing a critical review of techniques and protocols placed in context by authors with first-hand knowledge, and in many cases the authors have been instrumental in developing the approaches themselves. Over half the book consists of completely new chapters covering novel techniques and methodologies developed since the first edition was published, while all of the remaining chapters have been extensively revised and updated. The aim has been to present a comprehensive analysis of the pros and cons of each approach and its applicability, with experimental protocols provided where methodologies are not fully or straightforwardly described elsewhere. Each chapter also includes hints and advice from experienced practitioners and developers, handy tips that should make adoption of new methodology by a lab that much easier. The spirit of the first edition, which attempted to make yeast molecular genetics approachable for the newcomer, has again been retained by including chapters describing platform methodologies of strain construction and classical yeast genetics (Chapter 2), yeast transformation (Chapter 3), targeted gene manipulation (Chapter 4) and immunological approaches (Chapter 11). We hope that this will inspire sufficient confidence among non-yeast experts to encourage them to make forays into yeast territory when required, and perhaps, having read this volume, consider more extensive use of yeast in which to explore their areas of interest in what is still arguably one of the most powerful model eukaryotic systems.

The assembly of this volume has only been made possible by those yeast researchers, all experts in their fields, who gave generously of their time and patience. The broad geographic spread of contributors is a testament to the community-wide involvement with this book. We would like to thank all the authors of chapters in this volume for contributing with efficiency, speed and good nature. Finally, we thank those publishing editors at Elsevier who have been centrally involved with this project, Claire Minto and Lisa Tickner, for keeping the book as close as possible to schedule.

Ian Stansfield and Michael J. R. Stark
August 2006

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