Preface

It is now 10 years since the first edition of *YAC Protocols* was published in 1996. *YAC Protocols* was first produced to address the huge demand within the research community for a lab-based text that described in detail the wide range of uses for large insert yeast artificial chromosome (YAC) DNA clones. In doing this, the original editor, David Markie, and the many different contributors who provided descriptions of the protocols they used and developed, did a magnificent job. Indeed many of the techniques described within the first edition require little change and have stood up admirably to the test of time. Since the first edition, the use of YACs has proved invaluable for addressing a wide range of new biological problems ranging from those of basic biochemistry to assisting in the mapping and sequencing of the human genome.

The requirement for a second edition of *YAC Protocols* was prompted by a number of major advances in biology since the publication of the first edition. These advances have included the sequencing of the human genome, and the genomes of a wide variety of other organisms, and the increased use of transgenic animals for understanding the molecular basis of human and animal disease. In addition, since the publication of the first edition, the use of YACs for a variety of different applications have been replaced by the use of other large insert cloning vectors such as P1 and bacterial artificial chromosomes (PACs and BACs). PAC and BAC clones appear to address many of the perceived problems associated with YAC clones, such as the need for pulse-field gel electrophoresis purification of YAC DNA, chimerism, and instability. However, PAC and BAC clones can only harbor inserts of up to 200 kb, whereas YAC clones can hold up to 2 Mb of genomic DNA. Because of new evidence demonstrating the huge size of many genes in higher vertebrates (the term "gene" encapsulates all the exonic sequence and all necessary *cis*-regulatory sequences required for normal expression), the smaller capacity of PAC and BAC clones limits their use in the study of a potentially huge number of important genes. For this reason, YACs are still the cloning vehicle of choice when studying the characteristics of genomic fragments greater than 200 kb in length.

The intention of this second edition of *YAC Protocols* is not to completely replace the first edition, whose protocols, in many cases, are still relevant today, but to provide a much needed update on the new techniques currently being employed and to help redefine and illustrate the important roles still to be played by YAC technologies in the postgenomic age.

The first chapter, written by David Markie the previous editor of *YAC Protocols*, describes the very basics of yeast cell culture and outlines the different auxotrophic markers available and the recipes of basic media allowing for their selection in culture. Both Chapters 2 and 3 have been written by Cecilia Sanchez and Michael Lanzer and describe how YAC libraries can be generated from the genomes of novel species and pathogens whose genome sequences have yet to be sequenced (Chapter 2) and how, in the absence of extensive sequence data required for the design of PCR primers, these libraries can be screened for selected YAC clones by filter hybridization (Chapter 3). Chapter 4, by Sylvia Vasiliou and John Quinn, describes how an isolated YAC clone can be characterized further using restriction digestion, Southern blotting, nucleic acid hybridization, and PCR analysis to confirm the identity of the clone and to determine its integrity. Gaining access to, and analyzing, the huge wealth of mapping and sequence information currently available via the internet was an option almost undreamed of immediately after the publication of the first edition of *YAC Protocols*. In Chapter 5, Kerry Miller and Scott Davidson give a basic overview of how genomic sequence can be accessed and how this sequence can be rapidly analyzed using freely available and user friendly internet tools.

In Chapter 6, Shigeki Kawakami and coworkers describe a novel method of transforming DNA into YAC containing yeast clones using calcium alginate beads. In Chapter 7, Sanbing Shen describes how YAC clones can be altered to change different auxotrophic markers, a process known as retrofitting, and to allow amplification of the copy number of YAC clones within yeast cells. This ability provides advantages when attempting to isolate larger quantities of YAC DNA for a range of different procedures such as the production of YAC transgenic animals. In Chapter 8, Gabriela Loots describes how YAC clones can be conditionally altered by the engineering of *lox*P sites flanking target sequences within the clone. The flanking of these target sequences with *lox*P sites allows for their excision in vivo once the YAC clone has been successfully introduced into the genome of an animal transgenic for an inducible *Cre* gene.

Natalay Kouprina and coworkers describe a method that is likely to be the most important development in YAC techniques since their inception. In Chapter 9, Dr. Kouprina describes how novel YAC clones of a defined size and genomic content can be produced using homologous recombination (TAR cloning) in yeast. This technique has become especially relevant in recent years as a result of unparalleled access to multiple genome sequences via the internet. Furthermore, TAR cloning of YACs will promise to remove the specter of

chimerism that has represented one of the main perceived obstacles to the more widespread use of YAC clones.

In many situations, it may be helpful to manipulate YAC DNA clones either to make them smaller and more manageable or to join them together to produce a single "super-YAC." In Chapter 10, Yeon-Hee Kim and coworkers describe a simple and effective protocol that allows the controlled removal of large segments of YAC clones to produce smaller, more manageable, YACs. In Chapter 11, David Markie, Emma Jones, and Jiannis Ragoussis revisit and update a protocol that allows for the fusing of small YAC clones together into one large clone.

The production of an efficient targeting vector is frequently one of the most problematic steps encountered when attempting to carry out gene targeting (gene "knockout") in mouse embryonic stem cells. One of the main obstacles centers on difficulties encountered in the use of long range hi-fidelity PCR to isolate the long stretches of homologous sequences required to ensure the production of an efficient targeting vector. These problems include the insertion of replication errors that, despite what their name suggest, still affect hi-fidelity polymerases. These replication errors reduce recombination efficiencies and may even mutate the wrong part of the protein (a problem that would compromise the development of a conditional knockout model). Furthermore, the frequent inability to successfully amplify many long sequences owing to the presence of repetitive DNA is common. In Chapter 12, Peter Murray describes a protocol that circumvents these problems by allowing the production of highly efficient targeting vectors using YAC DNA. The ability to develop these vectors will greatly enhance the success of gene targeting in mouse embryonic stem cells.

The use of transgenic animals has become an important tool in understanding the role of genes in supporting health. Our added ability to produce transgenic animals using YACs has enhanced our understand of how large genes, or clusters of genes, and their regulatory elements contribute to the development and normal physiology of organisms and how changes within components of these genes can predispose individuals to disease. In Chapter 13, Alasdair MacKenzie describes a protocol that allows for the efficient recovery of intact YAC DNA and its subsequent microinjection into the pronucleii of one-cell mouse embryos. An alternative method of producing YAC transgenic animals is described in Chapter 14 by Pedro N. Moreira and coworkers, who have devised an efficient method of introducing intact YAC DNA into the mouse genome using intracytoplasmic sperm injection. This ground-breaking method complements, and may eventually supersede,

the use of pronuclear injection in the production of YAC transgenic animals.

The introduction and analysis of large genomic fragments in highly differentiated primary cell types such as those of the nervous system or the immune system has the real potential of facilitating a better understanding of the roles of genes in maintaining the differentiated phenotype and physiology of these cells. Furthermore, the use of large human genomic fragments to essentially humanize these cells will greatly aid in the design of more specific and efficacious drug therapies. However, getting these large genomic fragments into differentiated cell types and maintaining these cell types indefinitely has been problematic. Carl Anthony Blau and Kenneth Peterson have addressed many of the obstacles preventing the analysis of large genomic fragments in differentiated cell types. This has been achieved by first producing transgenic animals with YACs, deriving differentiated transgenic cells from these transgenic lines and immortalizing these lines using a novel strategy based on the introduction of crippled retroviruses. A detailed protocol outlining the procedures involved is described in Chapter 17.

The final two chapters in this updated version of *YAC Protocols* relates to the use of YACs in the mapping of genomes. YACs will continue to play a significant role in genome mapping as, although many genomes have been sequenced, many more important genomes, such as those of the myriad of pests and pathogens that still affect a distressing proportion of the human population, remain to be mapped. An essential aspect of mapping the geography of any genome is to understand the chromosomal location of particular genes and markers within that genome. A technique known as fluorescence *in situ* hybridization has been critical in developing detailed maps of different genomes. The use of YAC clones in FISH analysis is described by Thomas Liehr in Chapter 16. In addition, in Chapter 17, Marcia Santos et al. provide a detailed account of how YAC clones have been used to map the genome of the parasite *Trypanosoma cruzi*.

I am extremely grateful to all the authors who have enthusiastically provided these cutting edge protocols and I would like to acknowledge their help and support in realizing this new edition of *YAC Protocols*. We are in little doubt that any decline in the use of YAC-based techniques has stemmed from misconceptions of the difficulties involved in using YACs, such as the need to purify YACs using PFGE and their higher proportions of chimerism compared to PAC and BAC clones. However, many of the protocols described in this volume specifically address these problems. By gathering these protocols into one volume and demonstrating the enormous potential use of YAC technolo-

gies in a variety of different research situations, we hope that the use of YAC technology will continue to be considered by the wider research community as a viable tool in understanding the role of genetics in maintaining health, promoting pathogenesis, and conferring susceptibility to disease.

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