Preface

The exquisite binding specificity of antibodies has made them valuable tools from the laboratory to the clinic. Since the description of the murine hybridoma technology by Köhler and Milstein in 1975, a phenomenal number of monoclonal antibodies have been generated against a diverse array of targets. Some of these have become indispensable reagents in biomedical research, while others were developed for novel therapeutic applications. The attractiveness of antibodies in this regard is obvious—high target specificity, adaptability to a wide range of disease states, and the potential ability to direct the host's immune system for a therapeutic response. The initial excitement in finding Paul Ehrlich's "magic bullet," however, was met with widespread disappointment when it was demonstrated that murine antibodies frequently elicit the human anti-murine antibody (HAMA) response, thus rendering them ineffective and potentially unsafe in humans. Despite this setback, advances in recombinant DNA techniques over the last 15–20 years have empowered the engineering of recombinant antibodies with desired characteristics, including properties to avoid HAMA. The ability to produce bulk quantities of recombinant proteins from bacterial fermentation also fueled the design of numerous creative antibody constructs. To date, the United States Food and Drug Administration has approved more than 10 recombinant antibodies for human use, and hundreds more are in the development pipeline. The recent explosion in genomic and proteomic information appears ready to deliver many more disease targets amenable to antibody-based therapy. Without doubt, the continued use of antibodies in the 21st century is ensured by virtue of their powerful recognition properties and, as now demonstrated, by their successful partnership with protein engineering.

Antibody Engineering: Methods and Protocols presents cutting-edge techniques in antibody engineering research. In Part I, popular resources for antibody sequence analysis are described, together with in-depth discussions on antibody structural modeling. A directory summarizing useful websites relevant to antibody engineering is also included. Part II presents protocols for antibody lead generation from the cloning of immunoglobulin genes to the selection and generation of human recombinant antibodies by molecular display technologies and transgenic animals. For well-characterized murine antibodies with clinical potential, humanization by CDR grafting offers a proven solution to minimizing HAMA, while sparing the additional efforts in generating a completely new human antibody entity. Procedures are also described on reformatting anti-

body leads into monovalent, multivalent, and bispecific binding fragments for a wide range of in vivo applications. Part III focuses on the expression and optimization of antibody leads. Traditional antibody expression systems such as bacterial and mammalian cell culture are described, followed by more recent developments in insect cell cultures and transgenic plants. The use of plants is particularly important as it provides the scope for the mass production of antibodies at a fraction of the cost compared to conventional systems, hence making therapeutic antibodies more economical. Besides lead expression, chapters are also devoted to the in vitro affinity maturation of recombinant antibodies using phage display and a rational approach in the design of minimally immunogenic antibodies. Finally, Part IV details state-of-the-art technologies for the characterization of antigen-binding affinity and specificity. Some novel applications of recombinant antibodies in radioimmunotargeting, cancer immunotherapy, drug abuse, and the emerging field of proteomics are also presented. Although *Antibody Engineering: Methods and Protocols* cannot cover every facet of antibody engineering research, it is hoped that these chapters will provide the antibody engineer with the fundamental techniques upon which further imaginative technologies can be developed.

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