Preface

Over the past decades, monoclonal antibodies (monoclonals) have become invaluable for basic research, diagnostics, clinicians, and thousands of patients suffering from severe afflictions. Monoclonals are widely used laboratory reagents, and it is fair to say that the availability of a good monoclonal has led to increased understanding of the target biology as many experimental approaches, ranging from classical western blots to chromatin immnunoprecipitation assays, are enabled. Beyond basic research, therapeutic monoclonals are increasingly used as drugs and will account for over 50 billion USD in sales in 2013, and this figure is forecast to grow at double-digit rate, higher than any other therapeutic class. Since OKT3 (Muromonab-CD3, Johnson & Jonson/Ortho Biotech), the first monoclonal approved for human use, 34 other monoclonals have been approved. This is the tip of the iceberg, as it is estimated that over 400 monoclonals are in clinical development worldwide.

This brisk success is explained by several factors. First and most importantly, their specificity and low off-target toxicity often provide monoclonals with an exceptional "therapeutic window," i.e., the ability to dose them effectively with acceptable side effects. Monoclonals are also embraced because of their prolonged half-life and, more generally, as their pharmacokinetic properties are more predictable than for other classes of drugs. Consequently, the rate of success for the clinical development of monoclonals is significantly higher than for small molecule drugs or for other biologicals.

With the progress of genetic and protein engineering, academic labs and the biopharmaceutical industry have advanced many novel antibody formats and antibody-based approaches. These comprise multi-specific antibodies, fragments, antibody–drug conjugates, antibodies with enhanced effector function, and so on. However, despite these evolutions, being able to generate high-quality monoclonals against carefully selected epitopes remains the absolute foundation for subsequent improvements. Equally important is the meticulous characterization of candidate monoclonals. We actually contend that because of the greatly expanded toolkit for improving monoclonals, epitope selection and biochemical characterization has now become even more important for generating well-differentiated monoclonals. One can directly witness this notion at the bench with antibodies raised against the same target that perform very differently in various assays but also in the clinic, where monoclonals against different epitopes can exert very different responses in patients (e.g., the three anti-CD20 monoclonals Rituximab, Ofatumumb, and GA-101 by Roche, Genmab/GSK, and Roche respectively).

The purpose of this new edition of "Monoclonal Antibodies, Methods in Molecular Biology" is to offer modern approaches to indeed generate high-quality monoclonals against carefully selected epitopes, and meticulously characterize them. With a few exceptions, we deliberately concentrated on the basic IgG format. All the key steps from antigen generation to some final applications are described in these 36 chapters and should provide the reader with multiple useful methods to generate an appropriate monoclonal. We divided the book into four parts corresponding to four distinct objectives. Part I covers monoclonal

antibody generation, Part II deals with monoclonal antibody expression and purification, Part III presents methods for monoclonal antibody characterization and modification, and Part IV describes some applications of monoclonal antibodies. For each Part we strived to balance "must-have" protocols and recent innovative approaches, all "debugged" in the author's laboratories. In Part I, we included, for instance, protocols for the generation of monoclonals using natural sources such as mouse, rabbit, or immortalized human B-cells, but also in vitro selection methodologies such as phage and yeast display as well as antibody repertoire mining by deep sequencing. In Part II, several approaches are proposed for downstream purification of IgG as well as some alternative formats that should satisfy different requirements and downstream uses. In Part III, epitope mapping with various astute technologies such as phage- or bacterial display or extensive mutagenesis are well covered, as well as strategies for examining the primary sequence and structural and physicochemical properties of monoclonals. The latter are often overlooked but are important, as experiments performed with aggregated or unstable monoclonals can lead to erroneous conclusions. In Part IV, we provide some examples of use of monoclonals including immunofluorescence, crystallization chaperoning and the generation of solid-state arrays.

By no means is our selection of protocols exhaustive, a task impossible within the context of such a book. On the other hand, some topics are covered in more than one chapter, providing alternatives for the readers to select the most appropriate method for her/his use. We hope that our protocol choice will fulfill its intended goal of covering the crucial initial steps of monoclonal antibody generation and characterization with state-of-the art protocols.

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