METHODS IN MOLECULAR BIOLOGY™

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High Throughput Screening

Methods and Protocols, Second Edition

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Preface

In the 6 years since the first edition of this book, the field of high-throughput screening (HTS) has evolved considerably. In 2004, the Society for Biomolecular Screening (SBS) celebrated its 10th anniversary. The event and its timing were significant because SBS is the world's largest association of scientists, engineers and technologists associated with HTS. While the creation of SBS did not mark the birth of HTS by any means, its foundation in 1994 helped HTS find a common voice. It provided a discussion forum and a means to define and enforce standards. In 2006, SBS became the Society for Biomolecular Sciences, underlining the expansion of the members' interests beyond screening. Like any new technology, HTS went through growth stages. During the initial hype phase of the 1980s and 1990s, HTS, together with chemistry and genomics, was predicted to solve all of the pharmaceutical industry's pipeline problems. A fundamental change in drug discovery was afoot: the time-consuming physiology or medicinal chemistry experiments would be replaced by a numbers' game, made possible by screening large, combinatorially generated compound libraries against numerous genomically identified targets. While this approach did (and continues to) deliver, it fell short of the expected revolution, exposing it to criticism from within and outside the industry *(1)*. Learning from its mistakes, the HTS profession entered a period of change marked by an increased integration. The once stand-alone HTS groups matured into an essential, integrated component of the discovery effort. Contrary to the fears of many of our colleagues, HTS did not replace hypothesis-driven research but rather expanded it. In addition, because an HTS campaign is inherently expensive, more effort was expended to insure the quality of the hypothesis. Finally, compounds discovered by HTS enabled the testing of marginal hypotheses, thereby increasing the serendipity role in discovery.

To reach this maturity level, the HTS field had to learn to "play well with others." Of course, robotics, automation engineering, and data handling remain the hallmarks of HTS. But to be truly useful, HTS had to be integrated with the other discovery disciplines: genomics, molecular biology, cell biology, enzymology, pharmacology, and chemistry. Successful discovery starts long before and continues long after an HTS campaign. It also became clear that a large number of tests is not a replacement for quality components. Long gone are the days in which a marginally active target, or a target in a marginally relevant physiological state, is screened against large collections of compounds of questionable quality, diversity, or purity. Success is measured less by the number of compounds screened or by the hit rate and more by the quality of the chemical series entering the clinical pipeline. As a reward, HTS researchers can now point to several marketed drugs whose birth place was a well in a microtiter plate *(2)*. For example, in the breast cancer therapeutic indication alone, three drugs have been introduced, which originated from an HTS campaign and are worth mentioning here: (1) Iressa™, an ATP-competitive inhibitor of the epidermal growth factor receptor tyrosine kinase; (2) sorafenib tosylate or Nexavar™, a specific inhibitor of the kinase Raf-1; and (3) tipifarnib, or Zarnestra™, an inhibitor of protein farnesyl transferases.

With technology comes training. The preface of the first edition described how, at that time, "Nearly every scientist working in HTS had a unique story for how they came to be there," and that "All that is changing. Training programs are beginning to appear and the techniques created in HTS are being used more and more frequently in laboratories outside the field." Six years later, reality surpassed even the most optimistic predictions. Over 55 academic screening centers have been created *(3)*, which provide both HTS services and training. Universities have become a major player in this field, educating researchers who, in the past, had to rely on extramural institutions to learn the trade. The National Institute of Health Roadmap, created in 2002, has completed its first phase and created the Molecular Libraries Screening Center Network (MLSCN) as part of the Molecular Libraries Initiative *(4)*. These 10 HTS centers were established as a pilot program to apply HTS techniques in academic research with the overarching goal to "expand the availability and use of chemical probes to explore the function of genes, cells, and pathways in health and disease and to provide annotated information on the biological activities of compounds contained in the central Molecular Libraries Small Molecule Repository in a public database". Historically, serendipity and keen observation of natural events have been the main source for these tools. HTS now allows the systematic search for such probes. In addition, HTS allows a better understanding of the specificity of these compounds, an essential characteristic for their usefulness.

While much has changed, the core principles of HTS have largely remained unchanged. Each organization is structurally unique, but all retain key elements: an assay must be developed, a chemical library must be assembled and managed, a screen must be performed, and data must be analyzed. Each of these functions is discussed in this volume.

While assembling this new edition, we made a few choices. First, we wanted to remain true to the mission of the first edition: to serve as an introduction to HTS for scientists who are just entering the field, as well as providing enough details to be useful for scientists in established HTS operations. Second, while the HTS field regularly sees the introduction of new screening technologies, we wanted to give the lion's share of the volume to the well established methods. They are most likely to be widely used by the intended reader. Third, we wanted to give a detailed treatment of the activities that are immediately related to HTS: compound library management, data handling, and robotics. Finally, we purposely left out ancillary methods: natural compound selection, chemical diversity assessment, orthogonal assays, and ADME-Tox issues. These essential tools would have been underserved in this volume.

The reader will encounter terminology that is unique to HTS and has unique connotations in this industry. To assist with this problem, the Society of Biomolecular Sciences has assembled a glossary *(5)*. We encourage both experienced "screeners" and those new to the field to review these definitions.

We hope this manual will be of use to you and would like to acknowledge the authors who contributed to this manual: not only are they experts in their field, they are also great teachers who wanted to share their knowledge and enthusiasm for HTS.

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