现代生物技术前沿

PROTEIN MICROARRAYS

〔美〕M. 谢纳 著

蛋白质芯片

（影印版）

科学出版社
www.sciencep.com

现代生物技术前沿

PROTEIN MICROARRAYS

〔美〕M. 谢纳 著

蛋白质芯片

（影印版）

科学出版社

北京

图字:01-2005-0930

内 容 简 介

人类基因组大规模测序工作已经完成，随着以功能基因组学和蛋白质组学为主要研究内容的后基因组时代来临，蛋白质芯片作为检测蛋白质存在和运动变化的高效工具，将为本世纪的疾病诊断和治疗、新药开发、分子生物学、航空航天、司法鉴定、食品卫生及环境监测等领域带来一场革命。

生物芯片技术是一种高通量检测技术，它包括基因芯片、蛋白质芯片及芯片实验室三大领域。蛋白质芯片以蛋白质代替 DNA 作为检测目的物，比基因芯片更接近生命活动的物质层面，能直接测定蛋白质的相对水平及与其他分子的交互作用情况，以定量化的方式反映基因的活动情况，因而蛋白质芯片有着比基因芯片更加直接的应用前景。本书对蛋白质芯片技术进行了全面细致地阐述，包括技术原理、生产方法、表面化学、检测策略以及抗原、抗体数据分析，同时全书图文并茂，提供了许多生物学应用实例及实验草案。

本书知识全面，内容丰富，集合了蛋白质芯片技术的基础知识和前沿研究，实用性广泛，可读性强。适用于从事生物芯片研究开发以及生物信息学、蛋白质组学、基因组学、分子生物学、生物化学、细胞生物学、生物技术等相关研究领域的教学科研人员或技术人员参考使用，也可做为研究生教材。

书名原文:Protein Microarrays
Original English Language Edition Published by Jones and Bartlett Publisher, Inc. 40 Tall Pine Drive Sudbury, MA 01776

图书在版编目(CIP)数据

蛋白质芯片/(美)谢纳(Schena, M.)著.—影印本.—北京:科学出版社,2005

(现代生物技术前沿)

ISBN 7-03-015183-6

Ⅰ.蛋… Ⅱ.谢… Ⅲ.蛋白质-芯片-英文 Ⅳ.Q78

中国版本图书馆 CIP 数据核字(2005)第 021174 号

责任编辑:李 悦

责任印制:钱玉芬/封面设计:陈 敬

科学出版社 出版
北京东黄城根北街 16 号
邮政编码: 100717
http://www.sciencep.com

新蕾印刷厂 印刷

科学出版社发行 各地新华书店经销

*

2005 年 4 月第 一 版 开本:787×1092 1/16
2005 年 4 月第一次印刷 印张:30 1/2 插页:10
印数:1—3 000 字数:700 000

定价:65.00 元

(如有印装质量问题,我社负责调换〈环伟〉)

Preface

"Don't be so humble—you are not that great."
—Golda Mier

THE COLD ROOM is one of biochemistry's most formidable abodes, a stingy environment that can be as chilly metaphorically as it is thermally. Many a researcher has been drawn by the siren-like lure of classical protein studies, only to fall prey to a stark reality: what is true inside the cell cannot always be easily recapitulated outside the cell. Protein biochemistry abounds with cold room stories that span the gamut from glorious to hilarious, and I have a few of my own (read on).

Re-wind the reels back to the second year of graduate school at UCSF. The year is 1986 and the boss is Keith Yamamoto. There were no laptop computers, no cellular telephones, no commercial Internet or e-mail, and the human genome sequence was just a pipedream. You remember those days. Those were wonderfully nostalgic days both in and out of the laboratory, and arguably the golden days of transcription factor studies. Roger Miesfeld had just isolated the rat glucocorticoid receptor, a hormone-dependent gene regulator that quickly found its way to center stage in the race to understand how animal cells turn their genes on and off. With cloned receptor in hand, I set out to test whether glucocorticoid receptor function could be recapitulated in yeast cells, the idea being that fungi would allow us to test evolutionary conservation in eukaryotes, and forthwith unleash the "awesome power of yeast genetics" Guthrie-style on this unsuspecting animal gene if we were able to demonstrate functional conservation. Remarkably, the rat receptor sprang to life on the first attempt, producing a diagnostic blue color change in yeast cells expressing a β-galactosidase fusion and a broad smile on the face of a nervous young scientist. Conclusions? There were exactly four: (1) the cellular transcriptional apparatus had apparently retained function across a daunting 1 billion years of evolutionary time, (2) yours truly was incredibly brave to work for the hard-driving Yamamoto even in light of having rapid success in the laboratory, (3) yours truly was incredibly gullible to attempt such a ridiculous set of experiments with no obvious chance of a positive outcome, and (4) scientific success requires a perplexing combination of skill, intelligence, naïveté, passion, chutzpah, bravado, luck, childishness, and *je ne sais quoi.*

Receptor experimentation in yeast necessarily involved grinding up yeast cells, fractionating the proteins by denaturing polyacrylamide gel electrophoresis, transferring the proteins onto nitrocellulose, probing the immobilized proteins with a monoclonal antibody, and examining the filter to confirm the presence of the expressed rat protein. But "western blotting" can be tricky, particularly with heterologous proteins, which are notoriously unstable even if the yeast cells expressing the foreign protein have been engineered to remove the fungal protease genes. Such studies are also challenging because in spite of the grinding action of glass beads, yeast cells are surprisingly sturdy and orders of magnitude tougher than their mammalian counterparts. To maximize our chances of detecting the rat receptor protein, we decided to hedge our bets by moving as quickly as possible through the process. In the interest of speed, I quickly loaded up a bucket with ice, cold water, and about a dozen culture tubes containing yeast cells, and rushed off to the cold room.

Anyone who has ever worked in a cold room is familiar with the stainless steel floors common to such enclosures, as well as the damp environment and the frequent use of glycerol buffers designed to stabilize proteins during extract preparation (the sage protein biochemists among you probably know what's coming next). Instead of walking slowly and carefully into the cold room, I dashed in with great haste and promptly found a small puddle of buffer on the cold room floor. Cold steel, humidity, and glycerol conspired to produce something more slippery than ice, and before the former hockey player had time to react, he found himself flat on his back, sending a spectacular shower of ice, water, and yeast cells flying in every direction, including down the front of his lab coat. Dazed and disoriented, I spent a memorably miserable hour disrupting yeast cells while wearing a lab coat soaked with ice water. Only San Francisco summers and UCSF oral examinations were colder. Upon hearing about the mishap, a colleague in the lab spent the next few weeks referring to me a "cool guy" who really knew how to "chill out," phrases that many found funny, but I found less so!

An equally amusing story involved the same cold room, an unfortunate rodent, and a smattering of 20s-something tomfoolery. Not surprising, rat glucocorticoid receptor experiments necessarily involved the occasional use of rats, and apparently one such rodent attempted to transcend its model organism status by escaping into the cold room, only to meet its fate underneath an unsympathetic cold room refrigerator. Some time later during a routine cleaning, a colleague in the Yamamoto lab found the deceased rodent under the refrigerator, mummified but otherwise intact. He appropriately noted that such a find certainly added new meaning to the term "lab rat." After deliberating a few minutes, he decided that the best use of the dehydrated rodent was as a frisbee, a decision that sent him running from the cold room back into the laboratory for testing. He proceeded to fling the newly found rat mummy across the room, striking an unsuspecting lab mate who let out a shriek that sent the entire lab howling. Not to be outdone, the startled lab mate picked up the shriveled projectile and disappeared for about 5 mysterious minutes, only to return to the lab with the rodent suspended in a bucket of liquid nitrogen. He then fired up a cassette player blaring a favorite rock song, and flung the frozen rat back at the perpetrator, sending the mummy sailing across the lab once more. This time, the brittle object struck the lab floor with a crackle, sending small pieces of shattered rodent sliding in every direction. Embellished slightly, but all true, those were cold room stories for the ages!

Nearly two decades have passed since the early days of graduate school, and one is struck by the changes in high-level science. I sometimes pine for those days insomuch as the profession seems to have gotten much more serious over the years, perhaps because the tools are so much more powerful now and perhaps because so much more is at stake in terms of the fame and fortune that await scientists who are able to negotiate its sharp corners with dexterity. But in terms of the sheer thrill and excitement of doing science, science has never been richer and more exhilarating. And while we have lost some of the lighter moments and nostalgia that were more characteristic of decades past, and relegate such memories to lapses of introspection, there is probably no looking back, and rightly so. With the rise of microarray technology, we are unraveling the complexities of the human genome at an unprecedented pace, and unraveling such mysteries is affording safer medicines, better diagnostics, and a deeper understanding of the biochemical basis of development, disease, aging, behavior, and many other aspects of life. The latest manifestation of microarray technology extends our studies beyond genes and messenger RNA molecules to the ultimate purveyors of the genetic code, the proteins. It is this coveted class of molecules that provides the focus of a dynamic new book *Protein Microarrays*.

Protein microarrays are analytical devices that contain collections of proteins printed in rows and columns on glass substrates. The protein equivalent of DNA microarrays,

these tiny chips allow the massively parallel analysis of protein function in a highly miniaturized and automated manner. Tens of thousands of recombinant proteins, antibodies, synthetic peptides, and other protein derivatives can be used to examine labeled extracts from cells, patient sera, and other sources. Specific binding between target molecules on the chip and proteins in solution yields a quantitative measure of the proteins expressed in a biological sample. In one format, antibody microarrays provide specific binding reagents for protein expression monitoring, the logical extension being a chip that would allow the simultaneous measurement of every protein expressed in the proteome. In another type of assay, microarrays of recombinant proteins allow the identification of protein binding partners in a highly precise manner. Indeed, all of the fundamental activities of proteins carefully delineated using traditional approaches appear to be amenable to protein microarray analysis; protein-protein binding, enzyme-substrate catalysis, receptor-hormone recognition, target-drug binding, and so forth appear to work as well on chips as in solution. In essence, a protein microarray is a cold room on a chip (without the slippery floors and rodents!). Protein microarrays appear to be poised to replace many of the current in vitro diagnostic tests that rely on large reagent volumes and plate assays. This highly interdisciplinary technology also promises a new genre of tests based on genomic and proteomic information.

Jones and Bartlett Executive Editor Steve Weaver initiated this project by telephone, followed by a lively dinner at Spago in downtown Palo Alto where we finalized plans for the book. Steve and I have worked together successfully in the past, so the decision to take on this ambitious project was easy. Steve's intellect eclipses his physical stature (which is impressive because he barely fits in the passenger seat of a Porsche!), and I am indebted to Steve for all of his guidance and insight along the way. A dept of gratitude is also owed to the entire team at Jones and Bartlett including Rebecca Seastrong, Anne Spencer, Elizabeth Platt, Pam Thomson, Louis Bruno, Dean DeChambeau, and many others who have made this project enjoyable and rewarding.

I continue to build on the solid foundation provided by my incomparable scientific mentors Dr. Daniel Koshland, Jr., Dr. Keith Yamamoto, and Dr. Ronald Davis, and thank them for their nurturing and wonderful knowledge of protein biochemistry. I must also thank my publicist Paul Haje for insisting that I take on this project, and my colleagues at TeleChem International, Inc. for their remarkable expertise and innovative ArrayIt products. I also owe a special acknowledgement to my family and friends for their enduring kindness, and to René Schena whose love is an unwavering source of inspiration during these demanding projects.

Make no mistake that the contributors are the real heroes in *Protein Microarrays*. We were anticipating a total of twelve chapters for the book and received exactly twenty-four. The project expanded to twice its anticipated size, which is a testimony to the excitement in the field and to the energy and generosity of the contributors. By assembling two-dozen chapters from the top protein microarray laboratories in the world, we essentially guaranteed the success of the book before it was even published. It has been our experience that a compendium from the world's foremost authorities allows us to convey information and data that are virtually impossible to obtain from any other source. We are confident that *Protein Microarrays* will help usher in a new era in protein biochemistry, and I ask you to join me in embarking on this wonderful journey together!

Mark Schena
January 2004

FOREWORD

PROTEIN MICROARRAY TECHNOLOGY is an emerging and evolving science. Unlike DNA chips, on which interactions are based on Watson–Crick base pairing, protein-antibody interactions on protein chips are determined by complex associations between epitopes on the target protein and the antigen-binding site on the detection molecule. Individual protein-ligand pairs can possess widely different affinities. Proteomic microarrays require capture and detection molecules with high affinities and low dissociation rates so protein detection will be seen over a reasonable concentration range for the experiment. It may be necessary to screen several different molecules against the same protein to find those that do not cross-react with other similar proteins, or lack reactivity due to conformational changes in the protein target (denatured, aggregated, etc.) in the milieu to be analyzed. For all these reasons protein chips are much more challenging than DNA chips. Unlike gene microarrays, there are many different types of protein microarrays. Antibodies, aptamers, recombinant proteins, peptides, phage, even small molecular weight chemicals/drugs can be used as a bait molecule and/or detection reagent. Usually, the molecule is an antibody (forward phase approach) or the cellular lysate itself (reverse phase approach), which are immobilized onto the substratum and act as a bait molecule. Each spot contains one type of immobilized antibody or bait protein.

Protein microarrays pose a significant set of analytical challenges not faced by gene microarrays. The first serious obstacle is the vast range of analyte concentrations to be detected. Protein concentrations exist over a broad dynamic range (by up to a factor of 10^{10}). To make the analysis even harder, a low abundance analyte always exists in a complex biological mixture containing a vast excess of contaminating proteins. Imagine that the specificity of a detection antibody is 99%, but a cross reacting protein exists in a thousand fold (or greater) excess. For each analyte molecule detected there will be ten cross-reacting contaminating molecules detected, and the signal over background will be unacceptable. The second obstacle is the sensitivity barrier that exists because PCR-like direct amplification methods do not exist for proteins. Consequently, protein microarrays require indirect, and very stringent, amplification chemistries. Adequate sensitivity must be achieved (at least femtomolar range), with acceptable background. Moreover, the labeling and amplification method must be linear and reproducible to insure reliable quantitative analysis. Finally the amplification chemistry must be tolerant to the large dynamic range of the analytes and the complexity of the biologic samples. The biologic sample may naturally contain biotin, peroxidases, alkaline phosphatases, fluorescent proteins, and immunoglobulins, all of which can substantially reduce the yield or background of the amplification reaction. The third analytical challenge is that the clinical power of protein microarrays can only be realized if the technology can analyze very small amounts of input material. The analysis of cancer cells, for example within a core biopsy, may contain only a few thousand cancer cells. Assuming that many proteins of interest, or their phosphorylated counterparts, exist in low abundance, the total concentration of the analyte protein in the sample is obviously very low. Another major hurdle that needs to be overcome is the limited (but growing) availability of well-validated high affinity specific antibodies for the multitude of analytes that are under analysis.

The availability of high quality, specific antibodies or suitable protein binding ligands is the limiting factor, and starting point, for successful utilization of protein microarray technology. Prior to use on any microarray format, antibody specificity must be thoroughly validated (e.g. single appropriate sized band on western blot) using a complex biologic

sample similar to that applied and analyzed on the microarray. A significant challenge for cooperative groups, funding agencies, and international consortia, is the generation of large comprehensive libraries of fully characterized specific antibodies, ligands and probes. A major initiative of HUPO (Human Proteome Organization) is the production, and qualification of antibody libraries that will be made available to the scientific community. Lastly, there is no established set of reference standard reagents for each of the various components of protein microarray platforms. Without the establishment of reference standards, it will be difficult to interpret results across time and between laboratories and platforms. Accuracy and precision are paramount for protein microarrays, as they are for any assay regardless of the degree of multiplexed testing. Without the appropriate controls and calibrators, clinical implementation will be impossible.

* * *

The Food and Drug Administration (FDA) is interested in all aspects of genomics, including protein microarrays because we believe the products developed using this new and evolving science will have profound positive impacts on public health. FDA participation is two-fold. In the area of research, FDA has begun laboratory work to develop microarray tests including protein microarrays. In the area of regulation, FDA has begun to proactively consider how it can effectively ensure new diagnostic devices are able to reach the medical marketplace in a timely fashion. For the past four years the Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD) in FDA's Center for Devices and Radiological Health has been actively seeking an early dialogue with manufacturers of such tests as they develop their product for commercial marketing. Several dozen have accepted this invitation and come in to discuss new products and those under development.

Although this text is mainly focused on protein microarray techniques, it is clear that these technologies may be of important use in diagnostic testing in humans. In Chapter 7, Lebrun describes modeling the proteome to define proteins associated with Rheumatoid Arthritis (RA). His team has assayed approximately 4,000 relevant proteins and found 5 with a significant predictive value for RA. He also describes the use of printed antibodies and proteins as clinical markers for cancer. Seong notes in Chapter 8 the commercial availability of protein microarrays is currently limited but the future is bright. Novel biomarkers for diagnosis and therapeutic efficacy as well as an evaluation of drug candidates in preclinical and clinical stages are mentioned.

For many of these tests the FDA has already identified an efficient route to market via guidance documents. The guidance for submitting applications for tests for Rheumatoid Factor (RF) is found on the OIVD web page (http://www.fda.gov/cdrh/ode/rhuema.html), and for monitoring the recurrence of cancer in previously diagnosed and treated patients is found at http://www.fda.gov/cdrh/ode/tumor821.html. For the latter, industry input was most helpful in enabling the agency to develop this guidance.

FDA is striving to keep abreast of the developments in proteomics. The agency has concerns about both the standardization and reproducibility of the microarray chips. For reliable clinical use, obviously there must be reasonable and reproducible performance on the same chip, on different chips manufactured or used at different times, at different locations, and by different operators.

Both quantitative and qualitative results can be obtained from protein microarrays. In many cases protein microarrays can be compared to diagnostic tests now available (e.g. RF, PSA monitoring, bacterial identification). In other cases they may present new and higher risks and FDA might require comparison to an established clinical diagnosis or other yardstick for truth.

Dialogue between manufacturers interested in offering a new protein microarray as a diagnostic test in humans can be formal or informal. On an informal basis, manufacturers can contact the OIVD and participate in the ongoing office level educational staff college put into place to help inform and educate our regulatory work group. On a formal basis, manufacturers can submit protocols for formal evaluation through a process referred to as the pre-IDE process. In this process, the manufacturer provides FDA with the investigational study that is designed to evaluate the microarray's safety and effectiveness for its intended use. There is no charge for this service and FDA tries to maintain a 60-day turnaround time for review. At the end of the review, depending on the nature of the issues identified, a manufacturer may wish to meet with FDA for further discussion or clarification.

Based on its experience and regulatory goals, during the pre-IDE process the FDA provides feedback and suggestions to the manufacturer on how to obtain the most useful scientific information during the study evaluation of the accuracy of the microarray test. The FDA input does not guarantee a favorable outcome, however. That also depends on the design of the microarray itself. Because of this and because the pre-IDE is a non-binding process, manufacturers submitting protocols for review are not locked into a course of action. However, by providing study designs before they are performed and either following them closely or explaining deviations clearly, FDA is able to complete its review process in a streamlined and expedited manner that benefits both the agency and the device sponsor.

Another Center of FDA, the Center for Drug Evaluation and Research (CDER), has identified the use of valid biomarkers in the drug development process in a draft guidance (http://www.fda.gov/cder/guidance/5900dft.doc). FDA has identified tentative criteria for valid biomarkers as those measured in analytical test system with well-established characteristics and with an established body of evidence for test results. The agency continues to consider what criteria should be applied to determine valid biomarkers. This draft guidance at the present time does not specifically address protein microarrays, although many of the general portions can be applied to these products.

It appears that the FDA, industry, and academia have a common interest in the development and use of protein microarrays and of techniques for establishing microarray diagnostic performance. As this text indicates, there is a wide range of scientific talent and many ongoing projects being applied to the many issues integral to the successful implementation of this new area of science. We at FDA hope that the science will help drive our regulation of new products and that we will be informed and helpful partners in the process of the making protein microarray technology an integral part of future health care.

Joseph L. Hackett, PhD
Emanuel F. Petricoin, PhD
Food and Drug Administration
Bethesda, Maryland

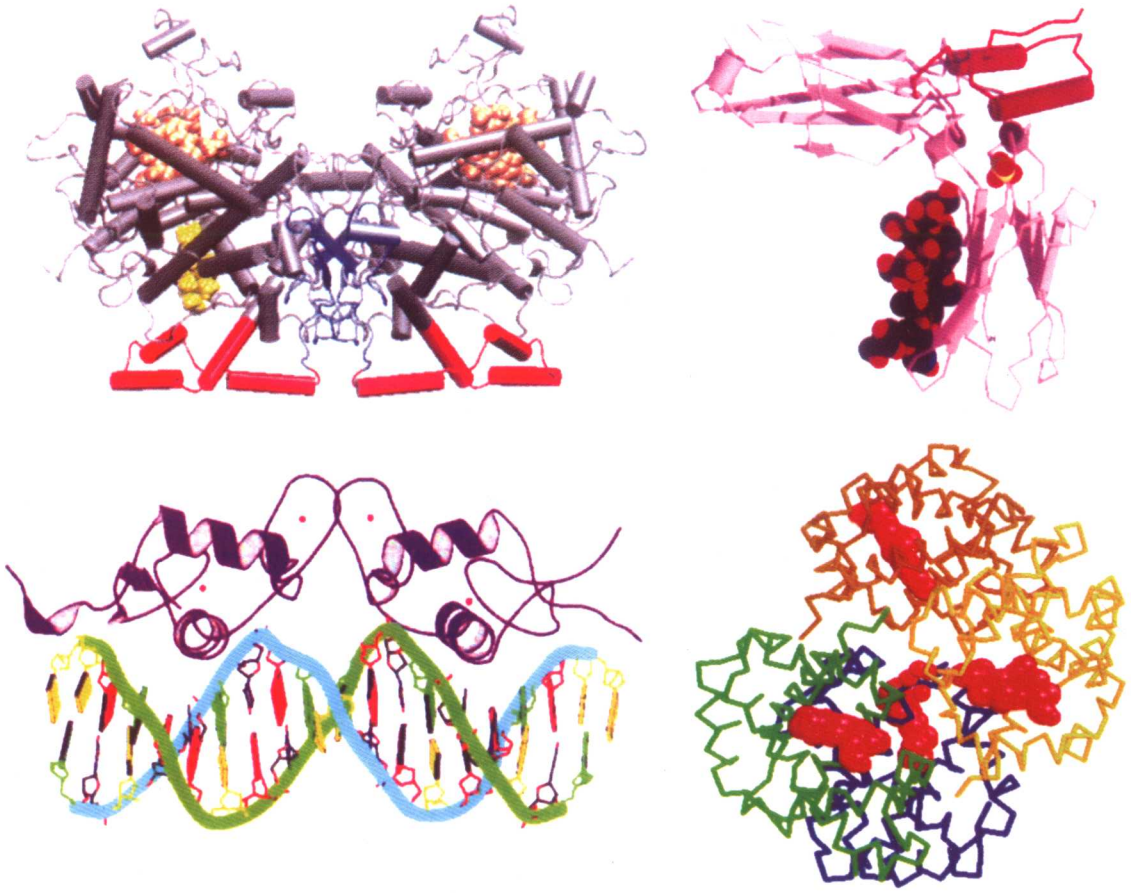

Figure 1.5

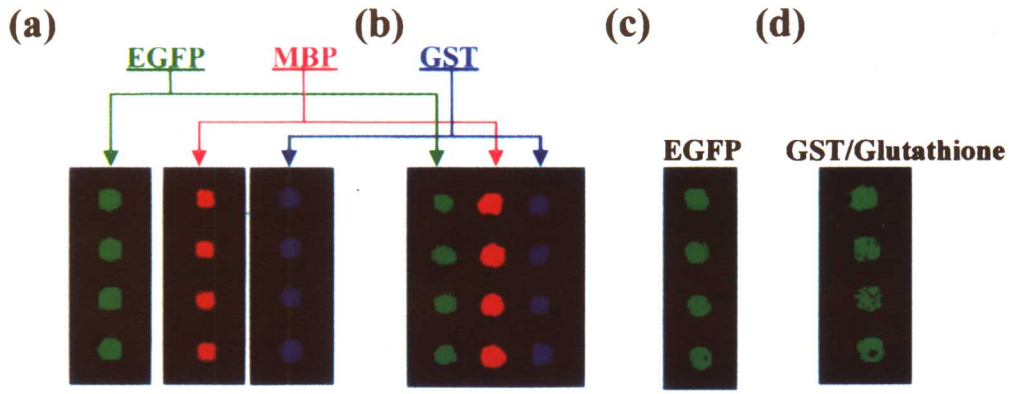

Figure 2.3

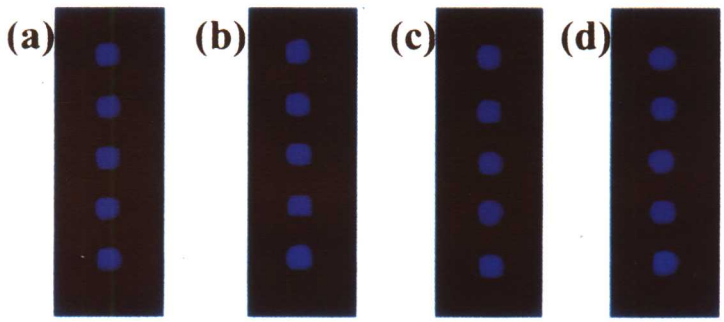

Figure 2.4

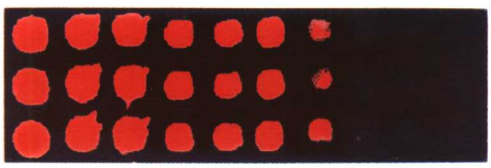

Figure 2.8

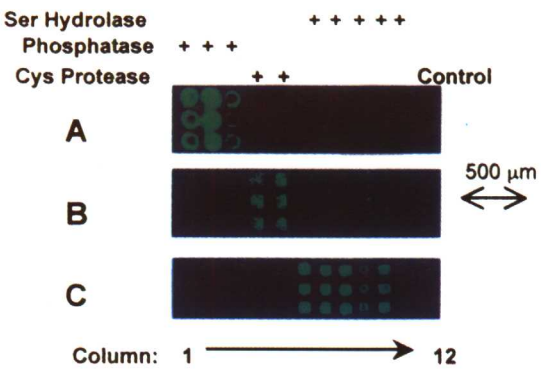

Figure 2.10

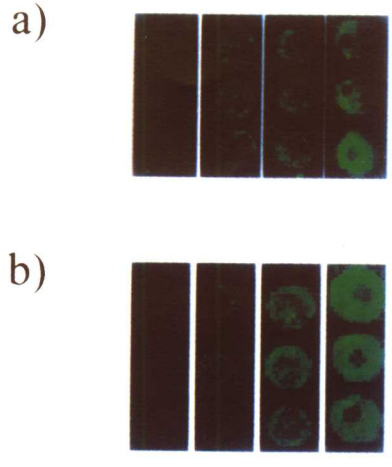

Figure 2.11

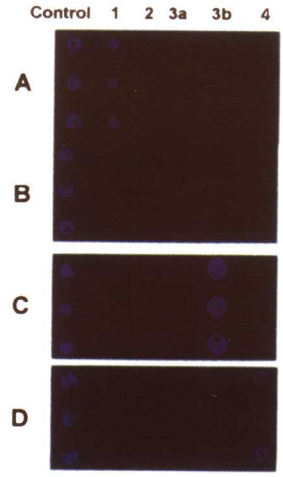

Figure 2.14

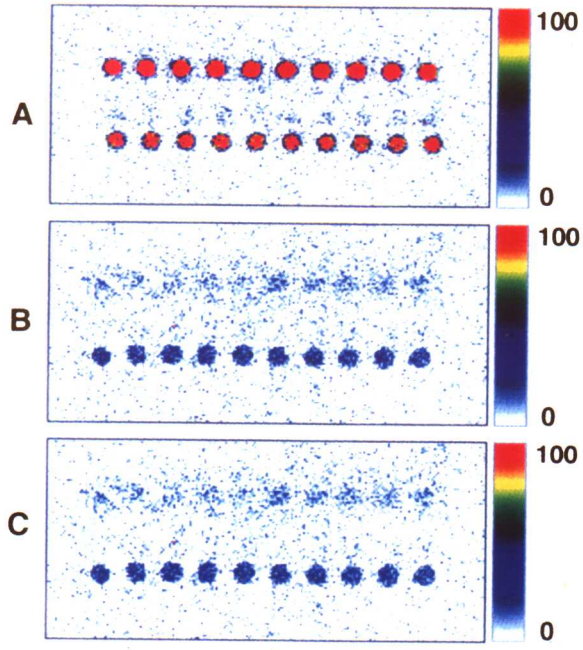

Figure 3.4

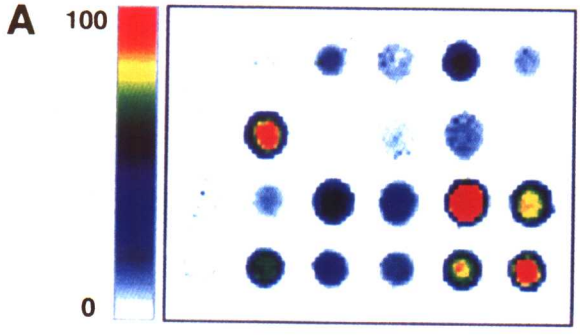

Figure 3.5

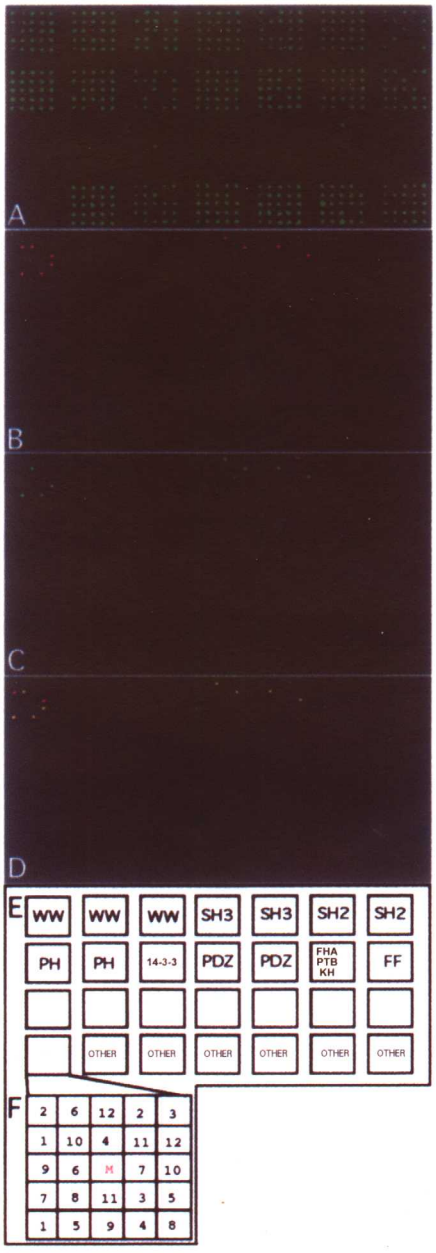

Figure 6.2

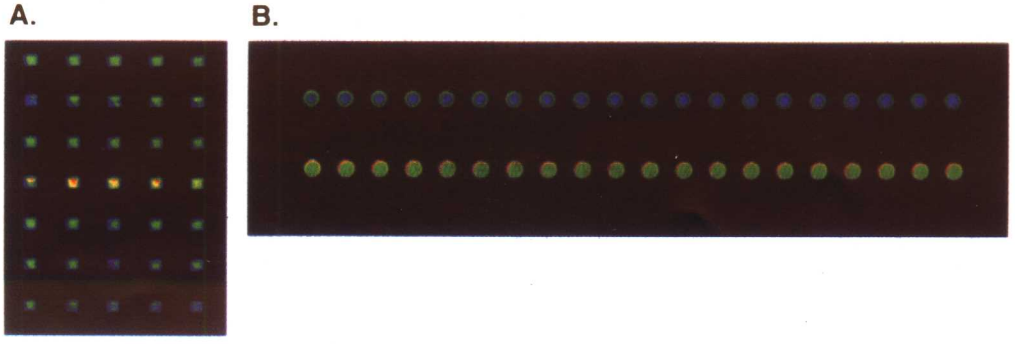

Figure 10.1

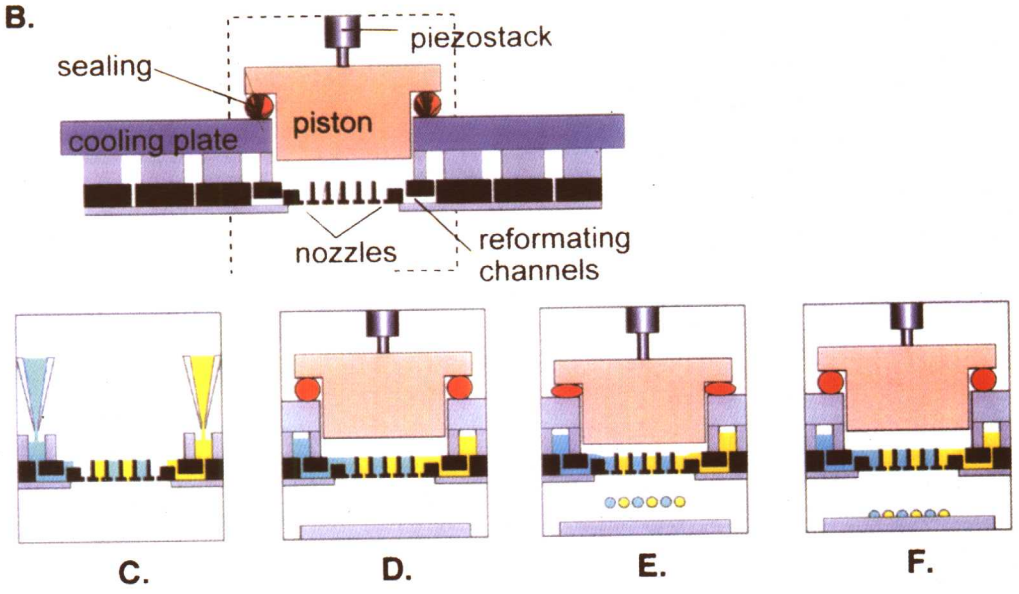

Figure 10.7

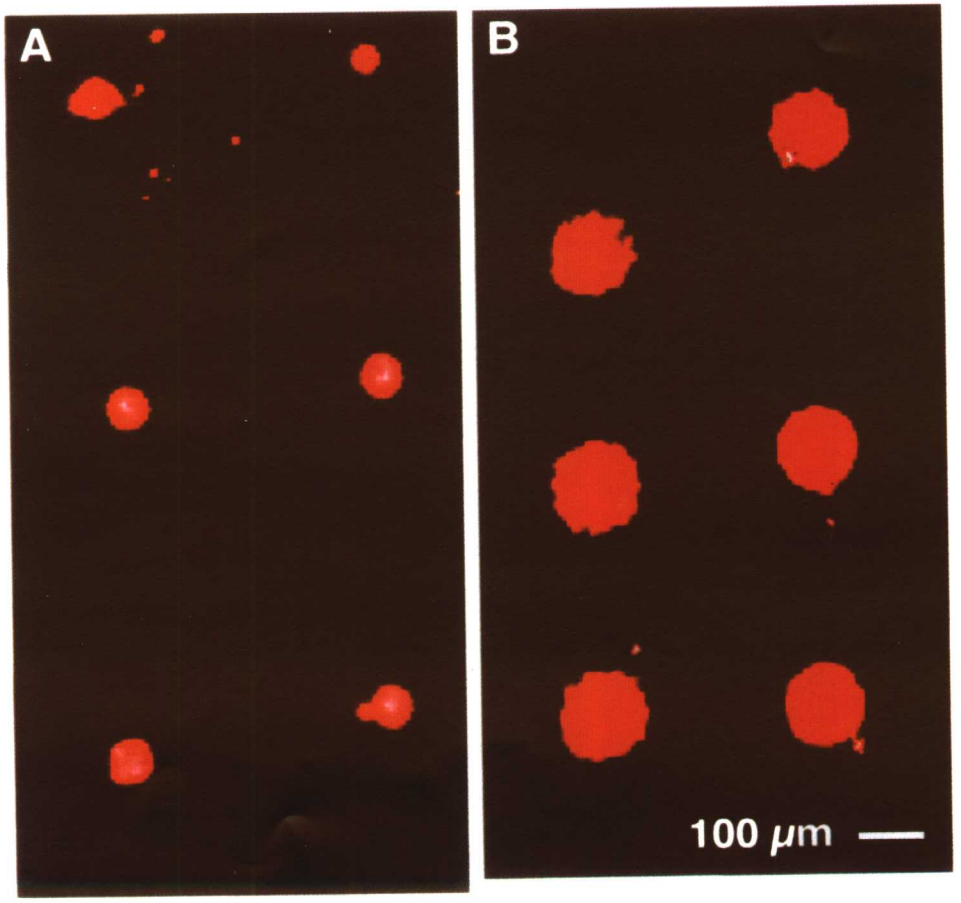

Figure 11.7

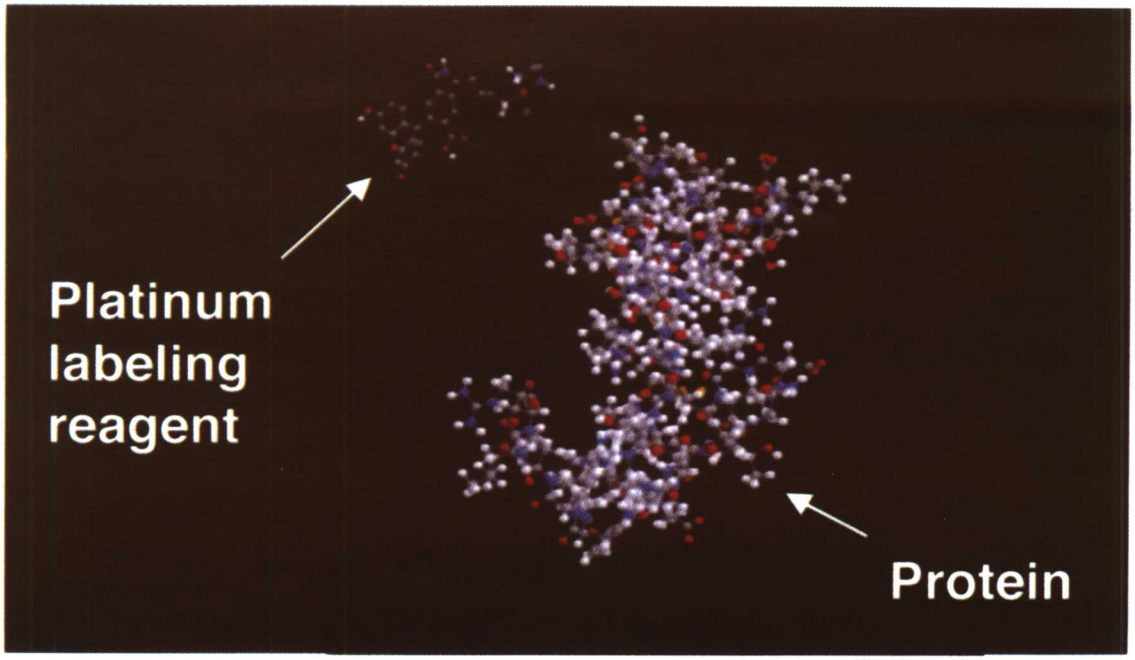

Figure 12.3

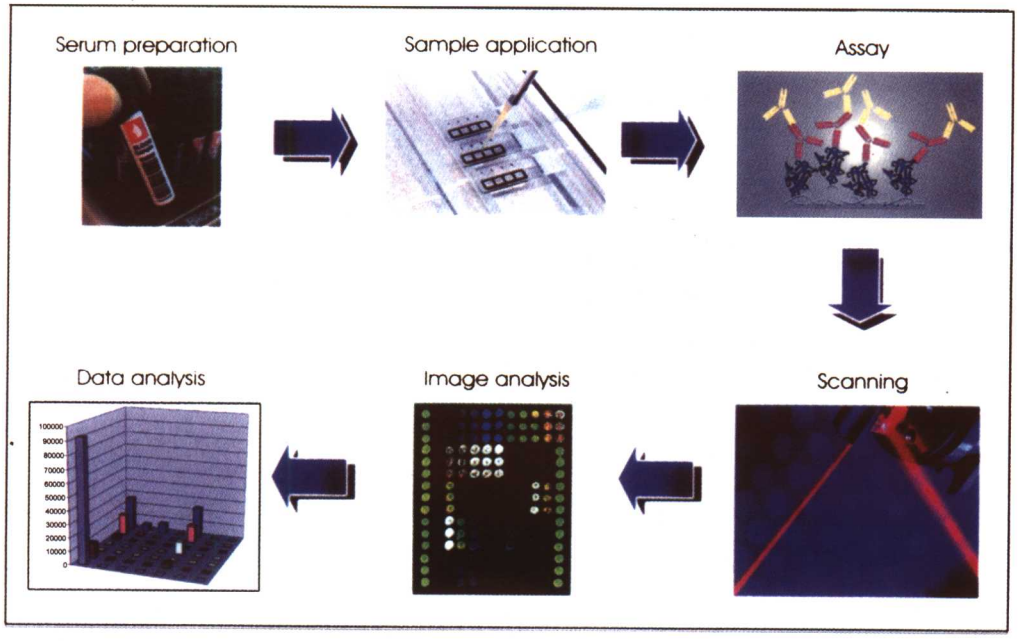

Figure 13.1

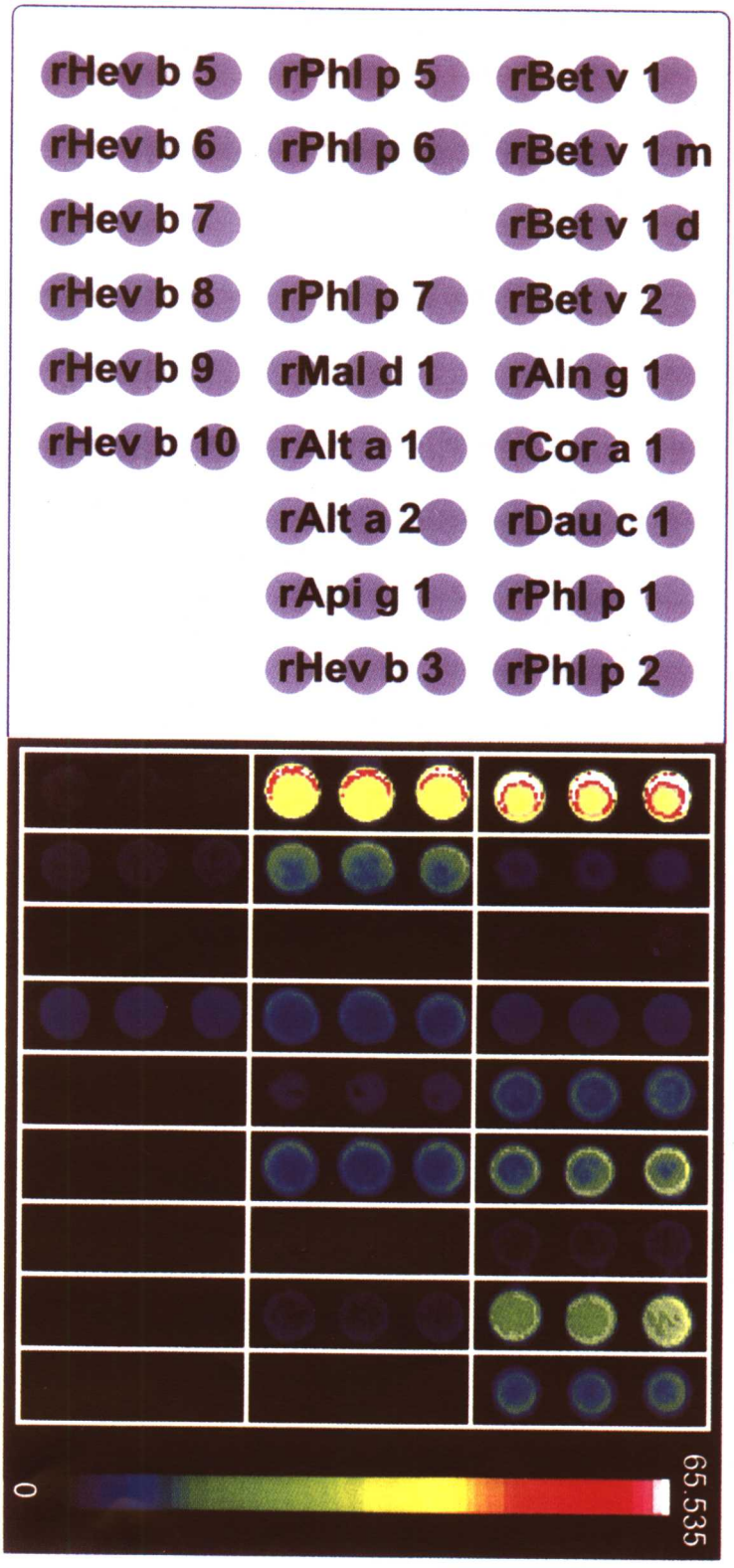
rHev b 5
rPhl p 5
rBet v 1
rHev b 6
rPhl p 6
rBet v 1 m
rHev b 7
rBet v 1 d
rHev b 8
rPhl p 7
rBet v 2
rHev b 9
rMal d 1
rAln g 1
rHev b 10
rAlt a 1
rCor a 1
rAlt a 2
rDau c 1
rApi g 1
rPhl p 1
rHev b 3
rPhl p 2
0
65.535

Figure 13.3

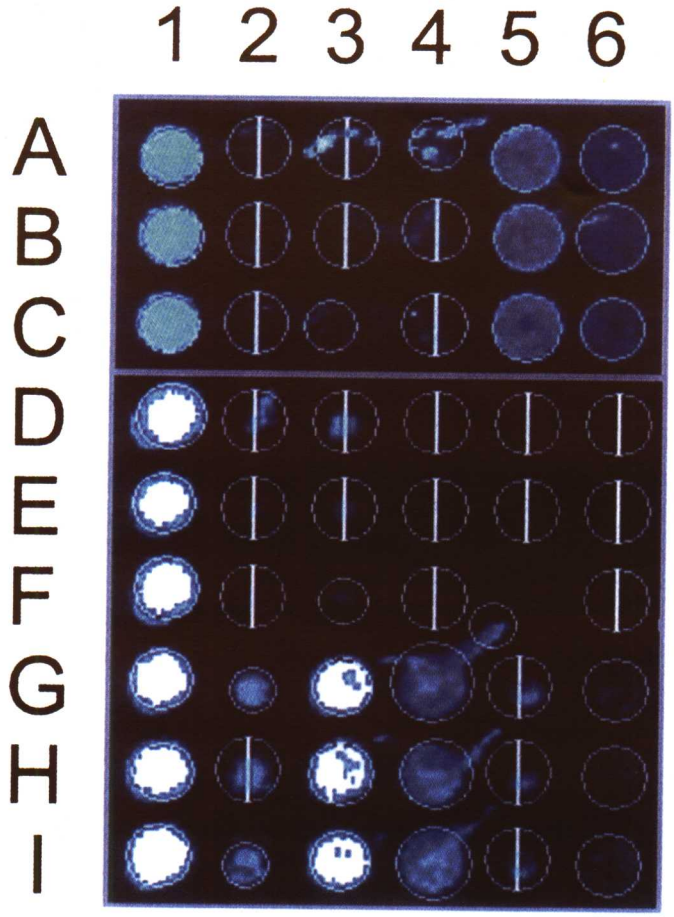

Figure 13.12