Preface

It is a rote of modern molecular biology classrooms to stress the importance of DNA–protein interactions by pointing out that species such as the bonobo and the human being share more than 95% of their genes – a level of similitude far exceeding that of, say, the translations of the *Odyssey* by Robert Fitzgerald and by Samuel Butler. The nonnegligible differences between our hirsute cousins and us, the argument goes, must find its explanation not so much in what genes we both use but in when and to what extent they are used. Hence the truly vital importance of understanding how regulatory proteins, be they active or passive, activating or inhibiting, interact with the genetic material to see it expressed in the proper way.

We say vital not only in the strictly intellectual sense of understanding the basic mechanisms of the living world (although that would seem to be a worthwhile goal in itself) but because our lives as individuals may well soon depend on an ever more precise understanding of gene expression. Following the discovery and widespread use of antibiotics in the mid-twentieth century, and the massive campaigns of vaccination that eradicated smallpox and helped control ancient scourges such as polio and tuberculosis, our species has recently become free, for the first time in its history, of the constant and urgent threat of infectious diseases. Next on the list of challenges to our health come problems associated with our recent extended lease on life, problems that are statistically rare in young people but tend to crop up in our later days. Type II diabetes, cardiovascular problems, neurodegeneracy, cancer, these are the new specters that haunt each of our visits to the doctor's office. These diseases have multiple etiologies and we have had some success in identifying and correcting some of their causes (be they environmental, related to our lifestyle or triggered by certain pathogens). In the long run, however, we understand that truly vanquishing them will demand a thorough understanding of the molecular mechanisms that turn cells immortal, that cause neurons to start dying, or that make cells insensitive to insulin. The same will hold true of multiple but less heralded pathological conditions associated with an improperly expressed genome, which are certainly as important to the person enduring them as cancer would be to the population at large.

Of course, the relevance of DNA–protein interactions is not limited to biology, medicine, or pharmacology. The word "biotechnology" coined by the Hungarian Karl Ereky in 1919 originally described the use of preexisting biological material for the creation of products and services but has come to signify much more in recent years. Gene splicing and the creation of transgenic species have fairly revolutionized agriculture, and in a world of roughly 7 billion mouths to feed, that qualifies as a life-altering realization in more ways than one. As we learn more and more about the interaction between DNA and the proteins that see to its proper expression in an ever-increasing number of species, we will be in a position to augment the benefits we reap from nature while at the same time minimizing our footprint on the environment. Whether this goal will be reached or not is another matter, but it is certainly easier to devise more efficient and environmentally friendly agribusiness strategies with a better understanding of how the world actually functions than relying on wild guesses and wishful thinking. Not content with modifying existing species, we have also taken the first steps in devising new life forms that may help,

for example, cleaning up oil spills; the success of such endeavors will rely heavily on our understanding of gene expression.

This is the third edition of *DNA–Protein Interactions: Principles and Protocols.* Each edition is separated from its predecessor by roughly 7 years, a period that appears to be sufficient for the field to come up with wild innovations in technology and its applications. New protocols are added to each successive edition, sometimes based on the novel use of ancient techniques, sometimes relying on new technological advances, but most of the time on some new integration of the two. These new ways to do things allow us to study where, when, and to what extent proteins interact with DNA, and that with an unprecedented precision and on a larger scale than ever. The current edition has retained updated versions of the basic techniques, the old-but-trusted ones that may never go out of fashion, but the impetus for this edition is the recent development of in vivo and genome-wide interaction techniques that have taken the domain by storm. The results obtained by years of in vitro techniques make everyone curious about whether they can be translated to an in vivo context, with DNA packaged in chromatin, and with several signaling cascades and protein actors all acting their part in unison. We are therefore happy to present several chapters on chromatin immunoprecipitation and other in vivo techniques. Along with new chapters on topological studies, photocrosslinking, FRET, and imaging techniques, we believe that this new edition will prove a worthy and practical addition to the series. As always, following the enlightened format of Methods in Molecular Biology, each protocol is rich in notes that authors think are important but which rarely make it into the spartan protocols that accompany most research papers. In the past, such notes were always our favorite part of these protocols, and we are glad to once again be able to exclaim "so that's how they do it" while perusing the notes section. It should be mentioned that some protocols featured in previous editions did not make it this time around, as they were either unchanged or perhaps less frequently used, but they are luckily still available on the SpringerProtocols.com database.

Although rapid progress in analytical capacities is something to be very proud of, we think it would be presumptuous to claim that the study of DNA–protein interactions has "finally come of age" following the recent (and sometimes spectacular) advances described in these pages. That is certainly not meant to belittle such amazing approaches as microarray-based gene expression analysis, atomic force microscopy studies, or ChIP-on-chip cartography, all of which were technological quantum leaps in the field and important enough to warrant the use of that overwrought cliché. It is meant, however, to emphasize the speed at which our fast-evolving technology allows us to ask new questions and to provide new answers. In a sort of biological application of Moore's law, we now live in a world where our ability to generate data on DNA–protein interactions and our ability to analyze it seems to increase exponentially, while the costs involved are getting low enough that experiments that were once the sole purview of big laboratories become available to most everyone. (Granted, we still have some work to do regarding the lowering of costs, but we are getting there.) The ingenuity of the scientific and engineering communities, driven by more and more demands from the medical and biotechnology fields, is bound to give rise to even more startling analytical technologies in the future, and personally I cannot wait to read further editions of this volume. I am sure that I will be as amazed then as I was this time around.

The current edition would not have been possible without the pioneering work of Geoff Kneale on the first edition, or without the expansion and diversification made by Tom Moss in the second edition. Both have my gratitude, and I thank Tom for bringing me aboard as coeditor for this third edition.

Have fun in the laboratory.

Benoît Leblanc