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978-0-521-88231-6 - The PCR Revolution: Basic Technologies and Applications

Edited by Stephen A. Bustin

Frontmatter

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THE PCR REVOLUTION

The invention of the polymerase chain reaction (PCR) won the Nobel Prize in Chemistry in 1994 and remains one of the most important scientific discoveries of the twentieth century. More than 50,000 researchers in the United States use PCR, and this is reflected in the thousands of publications using this technology. In this book, Professor Stephen A. Bustin, a world-renowned PCR expert, has gathered contributions that describe in detail the latest innovations and the overall impact of PCR on many areas of molecular research. The book contains personal reflections, opinions, and comments by leading authorities on the many applications of PCR and how this technology has revolutionized their respective areas of interest. This book conveys the ways in which PCR has overcome many obstacles in life science and clinical research and also charts the PCR's development from time-consuming, low throughput, nonquantitative procedure to today's rapid, high throughput, quantitative super method.

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The PCR Revolution

BASIC TECHNOLOGIES AND APPLICATIONS

Edited by

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Foreword

Russell Higuchi

Advances in science and technology come at an ever increasing pace. What was state of the art can be obsolete in just a few years. The old saw “The work that earns a Nobel prize today will be a graduate thesis ten years from now” may underestimate the rate of change. The hard-earned accomplishments of scientists and technologists can be made to seem trivial well within the time frame of a career. Faced with this, what do scientists celebrate as their accomplishments?

In the Stephen Sondheim play *Sunday in the Park with George*, about the pioneering artist, George Seurat, the character Dot says that the only things worth “passing on” to posterity are “children and art.” I agree wholeheartedly with the children part but I wondered about how broadly art could be defined in this context. As evidenced by university colleges still organized around “Arts and Sciences” – a holdover I believe from classical uses of the terms – science and art did not used to be so far apart. I believe that beautiful, inventive thinking can be art, and good science is full of beautiful, inventive thinking – such as PCR.

When I first heard of PCR, I thought – art. When I later joined Cetus and heard from Kary Mullis his idea to use a thermostable enzyme – art. When Kary proposed the Hot Start (a bit arcane but to someone who was now an aficionado) – art.

Nonetheless, I did find myself thinking, “PCR – five years and something even better will come along.” More than twenty years later, however, that has not proven to be the case, as PCR has matured and, as evidenced by the chapters in this book, is still increasingly useful. Part of that is due to real-time PCR, which I was the first to put into practice, and which is well covered in this book.

However, something better will definitely come along. We already have highly parallel sequencing of clonally amplified single DNA molecules with a throughput of a human genome a week. We are close to true single-molecule sequencing of a human genome a day. If ways can be found to parse this enormous throughput cost effectively among large numbers of samples, why use PCR and a probe to guess at what sequences might be in a sample when you can know everything that is there and at what frequency?

So the question: If its use is so transient, can it be art? I think so. With art in general, “usefulness” is not a measure of its import. How and why it comes

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into existence is. Hence the import of books like this, in which, unlike in journal articles, the how and why are told.

Lastly, I was pleased to see a chapter here on ancient DNA and PCR. The late Allan Wilson and I reported the first recombinant DNA cloning and sequencing of ancient DNA.¹ It was through trying to make this more efficient that I first learned PCR.² In referring to this work, a hard-core biochemist I knew said, “this is not science, it’s art.” Now I like to think it was a bit of both.

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Preface

We live in an age in which hyperbole has become so pervasive that it is difficult to find apt expressions for something truly exceptional. Furthermore, impatience, haste, and short attention span seem to be added hallmarks of our times, inviting technological bandwagon effects that briefly promise the earth, but then cannot deliver because the technologies were either conceived in haste without proper regard for technical and biological concerns or are superseded by the next technological “revolution.”

The polymerase chain reaction (PCR) has been around a long time now: certainly as US Patent 4,683,202 since 1987, as a practical tool since 1985,¹ and as a theoretical proposition since 1971.² A Google search for “polymerase chain reaction” throws up more than 1.3×10^7 results, roughly the same number as a search for “monoclonal antibody,” the other wonder technology in the molecular arsenal. Its conceptual clarity, practical accessibility, and ubiquitous applicability have made PCR the defining technology of our molecular age, with a three-letter abbreviation as distinctive as that of deoxyribonucleic acid (DNA). It has even made it to Hollywood, where the re-creation of dinosaurs in *Jurassic Park* was accomplished using PCR technology. The concept is so perfectly simple that the elemental scheme remains unchanged since its inception: two oligonucleotide primers that define converging sequences on opposite strands of a DNA molecule, a DNA polymerase, dNTP building blocks, and a series of heating and cooling cycles. This prompts the selection and enormous amplification of specific DNA sequences; consequently, the needle-in-a-haystack stumbling block is magically recast as a solution that creates a haystack made up of needles.

In contrast, the detection of amplification products has undergone, and continues to undergo, pronounced changes that have led the technology into new contexts and uses. The most dramatic, and dare I say revolutionary, innovation has been the invention of real-time quantitative PCR (qPCR), which in a flash has addressed many of the practical limitations associated with legacy, gel-based PCR.³ This adaptation allows detection of the accumulation of amplified DNA in real time after each amplification cycle. At its simplest, a qPCR experiment uses legacy PCR protocols, with the simple addition of a DNA intercalating dye, most commonly SYBR Green.⁴ When complexed with double-stranded DNA, the

dye absorbs blue light ($\lambda_{\max} = 488 \text{ nm}$) and emits green light ($\lambda_{\max} = 522 \text{ nm}$) that is easily detected using a qPCR instrument that combines a thermal cycler with a fluorimeter.⁵ At its most complex, qPCR can use a number of fluorescent dye-labeled probes to detect and quantify multiple targets in the same tube.⁶ Certainly, qPCR has been a requisite for the translation of PCR from pervasive research technology to practical process and has propelled progress in every branch of the life sciences, from agriculture to zoology; indeed it has created and sustains whole new sectors.

So, how to describe this technology and do it justice? Luckily, PCR speaks for itself through the vast numbers of applications, settings, and achievements that are unthinkable without this simple technique. This book attempts to tell the story of the PCR and to shine the light on some of the scientific advances that would never have happened without it. It presents personal views of authors from a wide range of backgrounds, pursuing an eclectic mixture of interests but united in their appreciation of the key role played by the PCR in their individual pursuits. Contributors include giants of the PCR field: Carl Wittwer, the “father” of qPCR instrumentation^{5,7-9} as well as the pacesetter behind numerous practical qPCR innovations^{4,10-39}; Mickey Williams, one of the original “Taqmen” and major contributor to further modifications⁴⁰⁻⁴²; Fred Kramer and Sanjay Tyagi, inventors of the ingenious molecular beacons and variants, as well as practical applications for them⁴³⁻⁵⁰; and Michael Pfaffl, major contributor and instigator of the drive toward more reliable and appropriate target quantification.⁵¹⁻⁵⁹ The essays of these exceptional individuals are complemented by a range of contributions that focus on practical applications of the technology. These range from Susan Burchill, who has explored the clinical potential of this technology,⁶⁰⁻⁷³ to the wonderful world of ancient DNA research.⁷⁴⁻⁸³ Their stories are all about the impact PCR has had on these and many other areas of molecular research. They aim to convey a flavor of the obstacles faced by life science and clinical researchers and how the unique properties of the PCR have been instrumental in overcoming these limitations. The book also aspires to chart the development of this technique from a time-consuming, low throughput, nonquantitative procedure to today’s rapid, high throughput quantitative super method. Reading through these chapters clarifies just how phenomenally powerful this technology is.

Sadly, something as commanding as this technology is also open to abuse and inappropriate use – which is also addressed within these pages. This is particularly distressing when it involves peoples’ health and highlights the need for constant vigilance when interpreting PCR-derived data. Opportunely, the year 2009 sees the publication of the first set of guidelines for researchers publishing PCR-based results.⁸⁴

Tempus fugit and PCR continues to evolve. I hope that this snapshot and the associated reflections of a group of individuals who have lived this technology will help to inspire others, and make them reflect that while a technology might be great, it is the individuals who craft and struggle with it that make it truly extraordinary.

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