The Polymerase Chain Reaction

From the Reviews–

"The editors have tapped many of the leaders in PCR innovation to explore their particular twists on the technique and to discuss its impact on their fields. The resulting chapters provide thorough examinations of basic and advanced PCR techniques, with a satisfying balance between theoretical analyses and observed results, and often include the type of anecdotal advice not found in journal articles. Mullis's preface has the stream-of-consciousness style of a newsy letter, and he introduces many of the authors with colorful, good-natured similes and personal anecdotes that amuse and add depth to the following chapters. Mullis tells a good story as he recounts his invention of PCR in 1983 and the subsequent patent travails."

– SCIENCE

"PCR The Polymerase Chain Reaction is not just a manual of techniques, but represents the considered experience of practitioners, some familiar with the use of PCR and some interested in extending its application to new areas...A chapter on nonbiological applications, using PCR as a product tag, was unique and extraordinary. Some contributions, such as one on infectious diseases, and another on genetics, plants and PCR, incorporate large numbers of publications and applications in their respective fields...Overall, it is a useful book and, for a variety of reasons, is unique among books in this field. I am delighted to have a copy."

– Trends in BioTechnology

The Polymerase Chain Reaction

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Editors

Foreword by James D. Watson

With 112 Illustrations

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Cover illustration: Based on a photograph taken by Kary Mullis in a museum in Cologne. "The mosaic had been taken from Italy by the Germans. I think it was from Pompeii. I saw a similar design still intact in its original location in Herculaneum but had no camera that day. Vesuvius covered both cities in 79 A.D. I would guess that the structure of DNA was probably worked out about two thousand years before Watson was born and therefore nineteen hundred and eighty-eight years before Crick. The Romans, however, seemed to think that atoms were square, and most likely the significance of the structure did escape their notice. Of course, this was long before Avery."

Foreword

James D. Watson

When, in late March of 1953, Francis Crick and I came to write the first *Nature* paper describing the double helical structure of the DNA molecule, Francis had wanted to include a lengthy discussion of the genetic implications of a molecule whose structure we had divined from a minimum of experimental data and on theoretical arguments based on physical principles. But I felt that this might be tempting fate, given that we had not yet seen the detailed evidence from King's College. Nevertheless, we reached a compromise and decided to include a sentence that pointed to the biological significance of the molecule's key feature—the complementary pairing of the bases. "It has not escaped our notice," Francis wrote, "that the specific pairing that we have postulated immediately suggests a possible copying mechanism for the genetic material."

By May, when we were writing the second *Nature* paper, I was more confident that the proposed structure was at the very least substantially correct, so that this second paper contains a discussion of molecular self-duplication using templates or molds. We pointed out that, as a consequence of base pairing, a DNA molecule has two chains that are complementary to each other. Each chain could then act ". . . as a template for the formation on itself of a new companion chain, so that eventually we shall have *two* pairs of chains, where we only had one before" and, moreover, ". . . the sequence of pairs of bases will have been duplicated exactly." The process of DNA replication of necessity *doubles* the numbers of DNA strands present and does so precisely as regards sequence. At that time, of course, we could offer no biochemical evidence as to how this process might be carried out. We recognized that the strands would have to be unwound and that this would be difficult to achieve without everything getting tangled, but our conviction that the structure was correct made us confident that evolution had found a solution to this problem. Another question we raised was whether an enzyme was required, once nucleotides had found their complements in the original chain, to join them, speculating that the template chain might itself act as the equivalent of an enzyme.

Over the next few years, experiments were carried out that confirmed our general scheme, so that by 1956 the decision to hold a Symposium entitled *The Chemical Basis of Heredity* was not only possible but made sense. At that meeting, Max Delbruck and Gunther Stent presented theoretical arguments showing the possibility of unwinding DNA, although we had to wait until the late 1970s before the proteins that carry out unwinding were isolated. One consequence of unwinding and replication as we envisaged it was that DNA replication should be semiconservative, and this was demonstrated convincingly by Mat Meselson and Frank Stahl in 1958. Also reported at the 1956 meeting were the first results of Arthur Kornberg, who was beginning to dissect the biochemical pathways that carry out DNA replication. While DNA polymerase I was later shown not to be the enzyme responsible for DNA replication in *E. coli*, the demonstration that what was thought to be an immensely complex process could be analyzed by a simplified *in vitro* system, marked a turning point in studying DNA replication.

Quite apart from its importance in DNA replication, base pairing came quickly to play an important technical role when it was realized that base pairing confirmed *specificity* on the interactions between the separated strands of a DNA molecule. This was demonstrated experimentally in the late 1950s, when Julius Marmur and Paul Doty were able to denature DNA and, on cooling the solutions slowly, recover molecules that were double stranded by physical and biological criteria. When hybrid DNA molecules were made by using DNA strands from different organisms, the degree of reassociation depended on how closely related were the organisms from which the DNA came. A little later, Sol Spiegelman with Ben Hall and Alex Rich independently showed that RNA and DNA strands would hybridize. Following the development of cloning in the early 1970s, hybridization became a powerful method for detecting sought-after sequences, especially with the development of Ed Southern's convenient technique. The critical importance of the specificity conferred by nucleotide sequence was exemplified in the 1980s when oligonucleotides, synthesized to be complementary to known sequences, began to be used as probes, so-called allele-specific oligonucleotides (ASOs). Thus in 1983, Savio Woo's laboratory reported the diagnosis of emphysema cased by mutations in α-1 antitrypsin by using oligonucleotides specific for the normal and mutant forms of the gene.

So by 1986, we could use *in vitro* systems to synthesize DNA with high efficiency, and because both DNA sequencing and oligonucleotide synthesis had become routine, we knew that oligonucleotides could be used as primers to direct the synthesis of specific sequences. But it was only through a phone call from Mike Botchan that I learned that these various elements had been combined in a simple fashion with extraordinary results. In early 1986, I was in the last stages of planning that year's Cold Spring Harbor Laboratory Symposium on Quantitative Biology, *The Molecular Biology of Homo Sapiens*. It was the 51st Symposium and it seemed right to begin our second 50 years with a subject that had changed out of all recognition in recent years and a topic of special interest to us all. Mike told me of Kary Mullis and the exciting work going on at Cetus, and I resolved to invite him to present his work at the Symposium. Although a paper had been published in *Science* in December of 1985, this was the first occasion on which the polymerase chain reaction was described in a public meeting. Kary's presentation was all that I had been led to expect and the excitement that it aroused was palpable, although my main recollection is his suggestion that because "G" and "C" are hard to distinguish in printouts of

sequence, "guanosine" should be changed to "wuanosine," "G" and "W" being easier to tell apart. This suggestion had the additional benefit, Kary claimed, of producing W–C base pairs, thus giving me a base, Francis already having his.

By 1987, although only a handful of papers using PCR had been published, its potential was evident and we decided to devote a Banbury Center meeting to the myriad novel applications being developed. The meeting, held in December 1988, demonstrated that the polymerase chain reaction now ranked with cloning and DNA sequencing as an indispensable tool in the molecular biologist's armamentarium. And not only has it become an indispensable tool, but PCR has provided new ways to approach a problem. For example, cloning of genes for olfactory receptors was achieved by using conserved sequences of G-protein-coupled, seven segment transmembrane receptors as primers for polymerase chain reactions performed on olfactory cells. It is estimated that these receptors constitute a new multigene family with several hundred members.

The field in which PCR has had the most extraordinary impact is human genetics. One beneficiary is the Human Genome Project, and indeed all the projects that are looking to complete the mapping and sequencing of the genomes of *Drosophila*, *C. elegans*, the mouse, *Arabidopsis*, and so on. It seems to me that the immensity of what has to be done would overwhelm us if we did not have the power of the polymerase chain reaction to assist us. How slow progress would be without direct sequencing, mapping using microsatellite repeats, and sequence tagged sites. It is perhaps no coincidence that one of the first public discussions of the proposal to map and sequence the human genome took place at the same Symposium where PCR was presented. But in a quite remarkable way for an esoteric technique, PCR has had an impact far beyond the confines of the research laboratory. The first paper on PCR dealt with the diagnosis of sickle cell anemia, and the rapid implementation of DNA-based diagnosis has continued to depend on and further exploit the simplicity and specificity of PCR in detecting mutations. The simplicity of the reaction will ensure the development of diagnostic tests that can be used to screen populations, and its specificity will be used to search for multiple mutations in a single reaction by using sets of primers.

All this has not been achieved without difficulties of a kind that we could well do without. Research scientists have been able to use PCR freely, but companies developing diagnostic tests have been hampered by uncertainties in what licensing restrictions they might be subject to, and clinical geneticists have been concerned that royalty fees will make the costs of PCR-based tests prohibitive. I had warned that this situation was not likely to be acceptable to a Congress concerned with health care costs or to a public seeking better health care. One year ago, Roche began to enforce strictly its patent for heat stable *Taq* polymerase, raising concerns that scientists pursuing research that uses large quantities of the enzyme will be unable to buy what is needed. However, first steps toward devising a more acceptable pricing policy are the very recent decisions by Roche to offer discounts to very large scale users (with extra reductions for designated genome centers) and to license two other companies to sell the enzyme. These are welcome moves, but the danger remains that with federal funding becoming increasingly scarce, a highly successful and desirable human genome research program and its medical applications may be delayed.

It is a pleasure to be able to end on a happy note. Even as I write this, Kary Mullis is at the Nobel Prize ceremony in Stockholm, sharing the 1993 Prize for Chemistry

with Mike Smith, developer of methods for site-directed mutagenesis. Thus PCR and mutagenesis join with DNA sequencing as techniques that have transformed the way scientists work, and prove once again that the golden age of molecular biology is far from over.

Preface

The polymerase chain reaction (PCR) has been employed extensively in the medical and biological sciences since it was formally introduced at the Cold Spring Harbor 51st Symposium on Quantitative Biology (Mullis et al., 1986) and it has repeatedly resulted in three complaints. The first is that PCR has made DNA research boring. Projects that formerly required some subtle deduction, clever manipulation, special insight, or good fortune are now within easy reach of anyone willing to assemble a few reagents and a cycler, and follow a well-worn routine.

The second has arisen as a minor lament from professional molecular biologists who on seeing for the first time the simplicity of PCR openly regret that they failed to stumble on it themselves. For the former the solution is obviously to do things using PCR that were next to impossible before and are now conceivable but difficult. As for the second, I can answer with an old Bob Dylan refrain, "Can I help it, if I'm lucky?" The third complaint comes from the medical diagnostics community and will be the subject of further comment presently.

Indeed, PCR has to a surprising extent transformed the way we do molecular biology. It has become an integral part of the DNA laboratory, and one that we would rather not do without even if it does make things at times a little like work. The computer seemed to wander into the world and from there into the biology laboratories just when the explosion of available DNA and protein sequence information would have put an otherwise uncomfortable strain on the limited number of graduate students and computationally inclined monks that could have been exploited for the task of organizing and analyzing this new kind of information. Curiously in the same almost too timely way PCR was discovered beside a buckeye tree near Highway 128 in Mendocino County (Mullis, 1990), just when the time was ripe for it to accelerate the assault on the awesome complexity of information in the macromolecular archives of life on earth.

Roger Penrose, in *The Emperor's New Mind* (Penrose, 1989), proposes that in the case of certain inventions, "where much more comes out of the structure than is put into it in the first place," or in the case of "an engineering innovation with a beautiful

economy, where a great deal is achieved in the scope of the application of some simple, unexpected idea, (the invention) might appropriately be described as a discovery rather than an invention."

I entertained thoughts like this about PCR, toying between discovery and invention, and as its uses and variations multiplied far beyond my grasp and it became in the households I visited not only a household noun but also a household verb, I settled on discovery. I was amused to come across the idea in the context of Penrose's chapter on mathematical truth with its implication that certain things may or may not be invented, but others were there already, and given time, would be discovered.

On the other hand now and then I read that PCR caused a revolution in molecular biology. Specifically, I know of two kinds of revolution in molecular biology. There is the kind where a band of angry, young, well-armed molecular biologists, having formented their plans in the chill, rarefied air of the UCLA winter symposia, meeting clandestinely on the slopes during the morning talks, and later in the darker corners of the bar while the poster sessions wind down, converge in the Spring on Bethesda, assault rifles and ugly unpatriotic slides on hand, to settle once and for all the issue of NIH post-doc stipends.

Then there is the other kind, referred to as a paradigm shift, or a retreat to the drawing board, when disappointing data can no longer be hidden or explained by old notions. New concepts become fashionable and new paragraphs have to be written for introductions to papers and grants. Usually there are a number of powerful elders in important places that have to retire or die before things get rolling. Like for instance, Maddox, who is aging at the same rate as everybody else, or Dan, who may take a little longer. It could happen here.

But I do not recall either kind of revolution here on account of PCR. New paragraphs and grants. But assault rifles? No. Paradigmatic shifts? I do not think so. It was just business as usual exploring genes. Things went faster and easier and the range of possibility expanded. Nobody had to die for PCR to be accepted. It was just a new tool. That it came out of the organic chemistry lab of a biotechnology company was interesting but not shocking. Things had begun to flow from industry to academia already to some degree. And chemistry had been gnawing at biology all century.

Being a simple little thing PCR tends to work its was into many studies. Everyone thinks of their own little twist to put on it to make it work for their own particular problem. If in any way at all, that is the way PCR has been remarkable. As the inventor, I like taking credit for all the adaptations, but this is getting a little ridiculous. Far too much has been done with it now to think that anyone, even the intrepid authors assembled here by François, Richard, and me, can really definitively describe it, much less take credit for it. There are too many papers out there, and like technical papers tend to be when there are a lot of them all in one stack and they aren't yours, become so tedious that no one would live through reading them all anyhow! This book hopefully will relieve the reader of some of this burden.

We have tried to find some people who have read a lot of them and have something unusual to offer from their own experience, and in the limited sense at least that the authors selected are the only people who have experienced having chapters in this book, we succeeded. That was a relief.

We have tried to organize the book in a somewhat logical fashion. There are two very distinct things that the polymerase chain reaction does. It generates a particular discrete DNA molecule that was not present to begin with, and it amplifies DNA molecules selectively. The first of these functions includes the search, cut-and-paste,

and append operations that support the analogy often made between PCR and a word processor.

The latter, amplifier function is a separate matter. We might have tried to divide the book along those lines, but alas, one never does one without the other, and both seem necessary in almost every application. It is part of the magic of PCR.

So we planned a set of chapters largely devoted to methodology and another set oriented to particular areas where PCR has been applied. There is a lot of overlap. Few strictly methodological people are working with DNA.

A refreshing exception is Carl Wittwer, from, strangely enough, the Pathology Department at Utah Medical School. I would have thought, Chemical Engineering at Cal Tech, but I knew otherwise. If I were you, I would read his paper, or have someone more technically competent explain it. Carl has thought about PCR in a way that very few others have, and his thoughts are crisp and practical. I have always known that a good physicochemical description of PCR would be very useful, but deriving one was over my head. Others have tried but not succeeded.

Gavin Dollinger has written an interesting chapter. He claims that "There are at least three reasons for wanting to tag a commercial object with a submicroscopic label." He fails to remind the reader directly of the scene in *Blade Runner* where Harrison Ford discovers the source of a fish scale he found in the apartment of a "replicant." The Chinese seller of fish who has what looks like a scanning electron microscope on the counter beside her cash register examines the submicron label on the scale and reports "No, Mr. Dekker, this not fish, this reptile; it artificial." PCR machines are cheaper than SEMs and the labels would be smaller. Gavin has written a good chapter, but he missed this key reference, and did not speculate on how long it might have taken to make this identification if the scale had been tagged with DNA. With the advent of scanning probe microscopes this may not be a theatrical issue.

Craig Tuerk describes something called SELEX that is a potential winner in the race to achieve the best rational process for creating high affinity binding agents for anything at all. There is a lot of competition in this area because this capacity is central to not only diagnostics, but therapeutics. The way Craig goes about it is also interesting from the point of view of the evolutionary theorist. He is in the running with Affymax, Selectide, a host of others, and those attempting epitope expression on the surface of lambda phage.

The Editors' Award for the chapter with the most intriguing name goes to Svante Pääbo. He has moved to Munich and he prefers old bones to new ones, preserved brains to currently active ones. He has been on TV a lot. We do not know what will become of him or the nice German lady he is sure to marry someday.

The award for the chapter produced on shortest notice goes to Skip Garner. I was under the impression for many months that Skip was warned that we wanted a chapter from him about the same time as we warned Perucho, McClelland, Heller, and Tullis all of whom, and François, live near La Jolla and are frequent guests in my house. So we do not know whose fault it was, but Skip started late. Skip is one of the smartest people I know. He began his career trying to control nuclear fusion. Somebody pointed him to it, and he said sure, no problem. Then somebody said, how about biology, again, no problem. Skip notices how easy it is to do things, and he is a valuable asset during a weekend in the country. I'm planning to read his chapter.

Manuel Perucho, the man from La Mancha, should get some award for listing over 200 references only two of which bear his name, while including at least one citation to two of the three editors of this book. Manuel, how about Gibbs? Using McClelland's AP-PCR method on his colorectal tumor samples, he is sticking his neck out

by looking at his experimental results without fear of what they declare. The article establishes the author's scholastic base in case someone might think him insensitive to convention; it is worth reading and lending to your friends to read.

A couple of years ago I went down to Louisiana to see Jeff Chamberlain get married. Jeff was in Tom Caskey's department at Baylor. In the 1960s Tom and I, and Tom's wife, went to the same high school in Columbia, South Carolina. I did not know Tom then; he left Dreher High School the year I arrived, but I met him about 20 years later at Cold Spring Harbor. It was 1986. I liked him right away. Sound of his voice maybe, but it could have been the quality of his curiosity. Hard to know. It took a couple of years before we diciphered our mutual origins. Our moms lived down the street from each other. Tom's chapter explains, for his mother's sake what he has been doing in Texas all this time. Jerry Lewis might go for it. Howard Hughes did. But Tom's mom is concerned that he has been too long away from home. Tom has this feeling that if something beneficial for public health is possible, then it should be done right away and that his post-docs ought to be doing it. In spite of how ridiculous it might sound it has worked more than it hasn't. They work nights in Houston.

Of the folks in Tom's lab, Jeff Chamberlain was one of my favorites. The wedding was in Lake Charles and the bride was a woman of striking beauty with a name like a boy. Joel. Her dad was an absolutely charming southern doctor who raised horses and peacocks on the edge of a bayou in Lake Charles. Marrying somebody who loves her dad, is a good way to insure that you'll be taken good care of. The sins of the father are often visited on the husband, and if they are few, the husband gets a good deal. The Catholic priest who administered the vows probably was unaware of this and delivered a fairly lengthy social worker kind of wedding chat that could have been entitled "Can This Marriage Be Saved." I got sleepy. A lot of modern clergymen seem to succumb to this temptation, citing statistics about broken homes and such right in front of the bride.

Nonetheless, it seems like Jeff has done alright. He is now in Michigan and is still living happily with Joel. His chapter in this book describes a really satisfying adaptation of PCR in which nine different amplifications are done simultaneously. It is not satisfying for the supply side people who would prefer the one-reaction, one-tube, one-shot-of-polymerase philosophy, but for the people who entertain nightmares concerning all the different genes that really could be checked in a newborn baby or a fetus, and for Jerry Lewis, who is happy to know that those crazy scientists have found a way to detect Duchenne Muscular Dystrophy before the boy is even out of the womb, multiplex PCR is really a neat trick. It was not easy, and Jeff explains how it happened in his chapter.

We all wish him and Joel a happy life together, but more than that, those of us who love this book have to thank Joel and Jeff for bringing Richard Gibbs and me together in Lake Charles that weekend. In fact I was in the back of a cab riding home from one of the more rowdy functions of the wedding with Richard Gibbs, when he slipped in some conversation about a book he had agreed to edit for Birkhäuser and then he flattered me, I think, and asked me to help him, and I was too naive to say no.

What a mistake. Books are no fun. You can never finish your part on schedule and you feel guilty for a long time until you finally do; and then you feel like you could have done a lot better job if you hadn't been rushed. There's no way you are going to come out of it feeling good. Maybe when you see it on your bookshelf. But then you read your chapter.

A few months later back in La Jolla, after letting Richard take advantage of me like that, I had the good sense to flatter François Ferré one night and convince him to become the third editor. In the jargon of the publishers, and by then Richard and I,

François was to be the working editor. And François did work! On the other hand Gibbs is from Australia, where you cannot remember anything much, even if you did make some promises once, because it is so far away by the time you get somewhere it does not matter. And blood is rushing to your head all the time from being upside down.

I didn't work on the book because I was busy.

So the book being actually published is because of François. And he is the person who failed to catch all the mistakes, and Gibbs and I trusted him to find them! I met his father once. He wears good boots, and makes good wine and rules over a bit of France near Poitiers where his family has lived for a long time and that contains a crumbling castle from the thirteenth century. The man would have been in the wrong century in any century. He is a dear, as is his son. What is it about François that would make you want to introduce him as your friend to your fiancé or your mother is hard to describe. It is not that he does not see the need to compromise. That could be a cold trait and François is warm like an orange hearth at Christmas with a big dog lying over it. He is not frantic. He is noble in the very best sense. Read his excellent chapter on quantitation written with his colleagues at Immune Response Corp. All this won't come out in his chapter. He's a professional.

Michael McClelland was the off-duty birdwatcher who cleverly thought up the technique of AP-PCR. That is what he called it, Arbitrarily Primed PCR. It was easy to do but hard to interpret. Michael prefers sitting in his office to standing in his lab and has always been a theoretician. It is well suited to Michael's style and he has exploited it gallantly. The technique has attracted a number of other practitioners and a plethora of new names. The ultimate status of the new names coincides with the likelihood that no one who independently named it did so after they had a fair chance to know that Michael had already. This contradicts the logic of the most plausible supposition that such a technique would not have been discovered twice, but it does not in itself question the honor of the gentlemen who gave it its second and third names. The fourth and fifth names are in some doubt. That AP-PCR just next door to Michael's lab stands for Absolutely Preposterous PCR sets one to thinking that it probably happened only once.

Rick Tullis, who resembles Santa Claus, and whose name has a nice ring to it, has provided us with an intriguing chapter. In his first paragraph, if I follow his logic correctly, I believe he states that "radioactivity" might be included in the category of "nonradioactive detection." I think he might be showing off his ultrapedantic capabilities just to see if anyone notices.

But he taught me how to ski, and how to play Donkey Kong, both at the same UCLA Symposium in Squaw Valley in 1981. The alternative would have been for us to go to the meetings and listen to the news from Michael Bishop, Harold Varmus and the rest of the troops out on the cancer front, and there was a big storm coming in, the slopes were about to be closed, some people were about to be killed in an avalanche, Jennifer Barnett was on her way up from Berkeley right in front of the storm. I was about to get down K-2 after only one week of ski instruction. So what the hell, Tullis is a joy, let him have his pedantry!

At the end of the book there are two chapters on nonscientific issues, one by Ellen Daniell, who followed PCR from Cetus to Roche, and one by me, who flew the coop early but came back for the trial.

Which brings us to the third complaint I mentioned at the start of this Preface. This was the very real complaint of the medical diagnostics community during the years between 1986 and 1992 concerning the restrictive commercial policies of the Cetus Corporation, which were perceived to be limiting the widespread practical applica-

tions of PCR. Jim Watson, who has endearingly not established his reputation in the world by remaining silent when his social sensitivities were aroused (see Foreword), put it rather bluntly to me in private during a gathering of genome enthusiasts at UCLA. I agreed with him completely and would quote him directly, but to challenge the reader's imagination will paraphrase his comments into my own sentiments on the subject. The relevance of all this is only historical since Cetus is now out of the picture. In July 1991 Hoffmann-La Roche bought the PCR Division from Cetus and what remained of the old Whale was folded into Chiron. How Hoffmann-La Roche will handle the brokering or commercial development of PCR remains to be seen.

An enlightened commercial policy for licensing the polymerase chain reaction, given an awareness of its almost universal utility in DNA diagnostics and worldwide interest in its immediate applications, would recognize that no lasting purpose could be served by arrangements to restrict access in any way to this new technology.

In my opinion a reasonable percentage of gross revenues from any company interested in applying PCR in the diagnostics marketplace, would have been an acceptable and expected mechanism whereby Cetus could have accomplished the corporate goals of its stockholders. The immediate results of the restrictive policies adopted by Cetus with regard to the use of PCR for detecting infectious diseases were adverse sentiment from within the diagnostics industry, words of advice from Jim Watson, and needless expenditure of research funds by government agencies and private companies to find a substitute for PCR. These funds could have rather supported development of practical PCR applications on which Cetus could have drawn royalties. But then something happened that ironically rewarded Cetus for its contrary position.

In the summer of 1989, in reaction to being denied access to PCR by Cetus, who had promised it all to Roche, DuPont challenged the validity of two Cetus PCR patents, the primary one being US 4,868,202 (Mullis, 1987) in a civil suit in the Northern California District federal court and also filed for patent reexamination with the Office of Patents and Trade Marks. All of this cost Cetus millions of dollars and a lengthy diversion for its managers, lawyers and scientists. But once the lawsuit was settled and the '202 was thoroughly validated, the very high profile of the battle that had been fought in the PTO and federal court had so enhanced the perceived value of PCR that Cetus was able to command a higher price for that fancy little piece of paper than had ever before been payed for a US Patent. Hoffmann-La Roche paid Cetus $300,000,000. DuPont had done Cetus a favor. Our last chapter is my summary of this trial.

Kary Mullis
La Jolla, CA

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