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## Preface

Since the discovery/invention of the polymerase chain reaction (PCR) in the mid-1980s, use of this technology has become so widespread that few researchers will progress far in their careers without exposure to PCR in one form or another. The robustness and exquisite sensitivity of PCR has led to the development of a multitude of protocols that exploit different aspects of this methodology. PCR has thus been used to analyze nucleic acids from all known phyla, and even some species extinct for hundreds of years. Disciplines as diverse as ecology, forensic medicine, food production, and archaeology employ PCR analyses as key components of population monitoring, genetic fingerprinting, quality assurance, and research. The place of PCR as a cornerstone of current and future scientific endeavor is beyond question.

Such has been the expansion in PCR methodologies that a revision of the previous edition of *PCR Protocols* dealing with PCR was considered essential. In undertaking this revision we were conscious that although much has changed, the fundamentals of PCR remain unaltered. Indeed in some areas, methods developed 5 or even 10 years ago remain as effective and useful as more recent developments. However, in other areas rapid progress has led to exciting new advances. We are also conscious of a “PCR generation gap,” whereby many of the early developments in PCR have passed into folklore. The usefulness of this second edition of *PCR Protocols* to all researchers will, we trust, be enhanced by the provision of an introductory section dealing both with the history of PCR and the legal and practical issues surrounding the development of PCR-based methods today.

Such is the diversity of the PCR methodologies currently available that it would be impossible to provide an exhaustive PCR manual that covers all aspects of the technology. Many of the sections we have included can be, and in some cases have been, expanded into separate publications. Therefore in producing *PCR Protocols* we have sought to select those techniques that form a core manual for both the PCR novice and the adept. The methods we have selected represent worked examples from many fields that can be reproduced and adapted for use within the reader’s laboratory. We have sought to provide both a primer to allow the reader to gain basic experience of different PCR techniques, and in-depth insight into a variety of the more complex applications of PCR. Each section has been introduced by an overview chapter whose aim is to provide researchers with an understanding of the applications and limitations of the techniques that follow. As with each volume in the *Methods in Molecular Biology* series, clear instructions for the performance of the various protocols is supplemented by additional technical notes that provide valuable insights into the working of the technique in question. Though often brief, these notes provide essential details that allow for a successful outcome.

We would like to express our gratitude to each of the contributors to *PCR Protocols*, 2nd edition, who have been patient over the period required to collate their contributions. Finally, thanks are owed to our wives, Dorothy and Muriel, for their patience during the long hours we have spent on this volume.

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## PCR Patent Issues

Peter Carroll and David Casimir

### 1. Introduction

The science of the so-called polymerase chain reaction (PCR) is now well known. However, the legal story associated with PCR is, for the most part, not understood and constantly changing. This presents difficulties for scientists, whether in academia or industry, who wish to practice the PCR process. This chapter summarizes the major issues related to obtaining rights to practice PCR. The complexity of the patent system is explained with a few PCR-specific examples highlighted. The chapter also provides an overview of the exemption or exception from patent infringement associated with certain bona-fide researchers and discusses the status of certain high-profile patents covering aspects of the PCR process.

### 2. Intellectual Property Rights

Various aspects of the PCR process, including the method itself, are protected by patents in the United States and around the world. As a general rule, patents give the patent owner the exclusive right to make, use, and sell the compositions or process claimed by the patent. If someone makes, uses, or sells the patented invention in a country with an issued patent, the patent owner can invoke the legal system of that country to stop future infringing activities and possibly recover money from the infringer.

A patent owner has the right to allow, disallow, or set the terms under which people make, use, and sell the invention claimed in their patents. In an extreme situation, a patent owner can exclude everyone from making, using, and selling the invention, even under conditions where the patent owner does not produce the product themselves—effectively removing the invention from the public for the lifetime of the patent (typically 20 years from the filing date of the patent). If a patent owner chooses to allow others to make, use, or sell the invention, they can contractually control nearly every aspect of how the invention is disbursed to the public or to certain companies or individuals, so long as they are not unfairly controlling products not covered by the patent. For example, a patent owner can select or exclude certain fields of use for methods like PCR (e.g., research use, clinical use, etc.) while allowing others.

There are an extraordinary number of patents related to the PCR technology. For example, in the United States alone, there are more than 600 patents claiming aspects

of PCR. Such patents cover the basic methods itself (originally owned by Cetus Corporation and now owned by Hoffmann-LaRoche), thermostable polymerases useful in PCR, as well as many non-PCR applications, (e.g., *Taq* polymerase, Tth polymerase, Pfu polymerase, KOD polymerase, Tne polymerase, Tma polymerase, modified polymerases, etc.), devices used in PCR (e.g., thermocyclers, sample tubes and vessels, solid supports, etc.), reagents (e.g., analyte-specific amplification primers, buffers, internal standards, etc.), and applications involving the PCR process (e.g., reverse-transcription PCR, nested PCR, multiplex PCR, nucleic acid sequencing, and detection of specific analytes). This collection of patents is owned by a wide variety of entities, including government agencies, corporations, individual inventors, and universities. However, the most significant patents (*see Table 1*), covering the basic PCR method, the most widely used polymerase (*Taq* polymerase), and thermocyclers, are assigned to Hoffmann-LaRoche and are controlled by Hoffmann-LaRoche or Applied Biosystems (previously known as PE/Applied Biosystems) and are available to the public through an intricate web of licenses.

### 3. Navigating the PCR Patent Minefield

The following discussion focuses on issues related to the earliest and most basic PCR-related patents. A full analysis of the hundreds of PCR-related patents is not practical in an article this size, let alone a multivolume treatise. It is hoped that the following discussion will provide a preliminary framework for understanding the broad PCR patent landscape.

The early PCR patents now owned by Hoffmann-LaRoche have been aggressively enforced. In particular, the earliest patents intended to cover the basic PCR method and the *Taq* polymerase enzyme (U.S. Patent No. 4,683,202 to Kary Mullis, U.S. Patent No. 4,683,195 to Kary Mullis et al., U.S. Patent No. 4,889,818 to Gelfand et al. and foreign counterparts) have regularly been litigated and used to threaten litigation, even against academic researchers. This aggressive patent stance has created an environment of fear, confusion, and debate, particularly at universities and among academic researchers. Because of this aggressive patent enforcement, issues with respect to these patents are most relevant and are focused on herein.

#### 3.1. Obtaining Rights to Practice PCR

In the case of the early PCR patents, Hoffmann-LaRoche, directly and through certain designated partners, has made PCR available to the public under specific conditions, depending on the intended use of the method (*see* <http://biochem.roche.com/PCRlicense.htm> for availability of licenses and current details). For example, for nonsequencing research use, PCR users have two options. They can individually negotiate a license from Applied Biosystems (a proposition that is impractical for many researchers). Optionally, they can purchase “certain reagents” from a “licensed supplier” in conjunction with the use of “an authorized thermal cycler.” This essentially means that the user must purchase thermostable enzymes and thermocyclers from suppliers licensed by Hoffmann-LaRoche or Applied Biosystems. Not surprisingly, the price of these products from licensed suppliers greatly exceeds the price of equivalent products from nonlicensed suppliers. Indeed, thermostable enzymes from licensed suppliers may

**Table 1**  
**PCR Patents**

U.S. patent number	Issue date	Expiration date	Related international patents	Claimed technology
4,683,195	07/28/87	03/28/05	Australia: 591104B Australia: 586233B Canada: 134012B Europe: 200362B Europe: 201184B Europe: 505012B Japan: 2546576B Japan: 2622327B Japan: 4067957B Japan: 4067960B	Amplification methods
4,683,202	07/28/87	03/28/05	Same as 4,683,195	Amplification methods
4,965,188	10/23/90	03/28/05	Australia: 586233B Australia: 591104B Australia: 594130B Australia: 632857B Canada: 1340121B Europe: 200362B Europe: 201184B Europe: 237362B Europe: 237362B Europe: 258017B Europe: 459532B Europe: 505012B Japan: 2502041B Japan: many others	Amplification methods using thermostable polymerases
4,889,818	12/26/86	12/26/06 (currently unenforceable)	Australia: 632857B Europe: 258017B Japan: 2502041B Japan: 2502042B Japan: 2719529B Japan: 3031434B Japan: 5074345B Japan: 8024570B	Purified <i>Taq</i> polymerase enzyme
5,079,352	01/07/92	01/07/09	Same as 4,889,818, plus Europe: 395736B Japan: 2511548B Japan: 2511548B	Recombinant <i>Taq</i> polymerase enzyme and fragments
5,038,852	8/13/91	08/13/08	Australia: 612316B Australia: 653932B Europe: 236069B Japan: 2613877B	Apparatus and method for performing automated amplification

cost more than twice as much as from nonlicensed suppliers (1). This elevated cost can place a substantial financial burden on researchers who require heavy PCR usage, particularly academic researchers on fixed and limited grant budgets. To the extent universities require their researchers to use licensed products, the aggregate cost increase for many large research universities is substantial. (For a list of *Taq* polymerase suppliers and prices, including licensed and unlicensed suppliers, see Constans, ref. 2).

### **3.2. Bona-Fide Researchers Are Not Infringers**

As mentioned previously, Hoffmann-LaRoche has taken the position that academic researchers are infringers of their patents if they are not meeting the prescribed licensing requirements (e.g., not purchasing authorized reagents and equipment). At one point, several years ago, Hoffmann-LaRoche specifically named more than 40 American universities and government laboratories and more than 200 individual scientists as directly infringing certain patents through their basic research (3). Voicing the view of many researchers, Dr. Arthur Kornberg, professor emeritus at Stanford University and Nobel laureate, has stated that the actions by Hoffmann-LaRoche to restrain the use and extension of PCR technology by universities and nonprofit basic research institutions “violated practices and principles basic to the advancement of knowledge for the public welfare.”

Fortunately for academic researchers, the laws of the United States and other jurisdictions agree with Dr. Kornberg. US patent law recognizes an exemption or exception from infringement associated with bona-fide research (i.e., not-for-profit activities). The experimental use exception to the patent infringement provisions of US law has its origins in the notion that “it could never have been the intention of the legislature to punish a man, who constructed...a [patented] machine merely for philosophical experiments...” (4). An authoritative discussion on the research use exception appears in the case *Roche Prods., Inc. v Bolar Pharmaceutical Co.* (5). Even though this case is generally considered to restrict the scope of the research use exemption (failing to find noninfringement where the defendant’s acts were “solely for business reasons”), the case makes it clear that the exception is alive and well where the acts are “for amusement, to satisfy idle curiosity, or for strictly philosophical inquiry.” Thus, to the extent that researchers’ use of PCR is not applied to commercial applications or development (e.g., for-sale product development, for-profit diagnostic testing), the researchers cannot be considered infringers. For example, pure basic research, which describes most university research, cannot be considered commercial, and the researchers are not infringers. This applied to the PCR patents, as well as any other patent. Hoffmann-LaRoche has taken the position that “These researchers...are manifestly in the business of doing research in order to...attract private and government funding through the publication of their experiments in the scientific literature, create patentable inventions, and generate royalty income for themselves and their institutions through the licensing of such invention.” However, current US law does not support this extraordinarily broad view of commercial activity, and Hoffmann-LaRoche seems to be alone in making such broad assertions.

Although the above discussion relates to the United States, researchers in other countries may or may not have the same exemption. The scope of this article does not permit a country-by-country analysis. However, it must be noted that many countries

are in alignment with the position taken by US courts or provide an even broader exemption. For example, it is not considered an infringement in Canada to construct a patented article for the purpose of improving upon it or to ascertain whether a certain addition, subtraction, or improvement on it is workable. The Supreme Court of Canada spoke on this issue stating that “[N]o doubt if a man makes things merely by way of bona fide experiment, and not with the intention of selling and making use of the thing so made for the purpose of which a patent has been granted, but with the view of improving upon the invention the subject of the patent, or with the view of assessing whether an improvement can be made or not, that is not an invasion of the exclusive rights granted by the patent. Patent rights were never granted to prevent persons of ingenuity exercising their talents in a fair way.” Likewise, UK law provides an exemption from infringement for acts that are performed privately and for purposes that are not commercial and for acts performed for experimental purposes relating to the subject matter of the invention. The experimental purposes may have a commercial end in view, but they are only exempt from infringement if they relate to the subject matter of the invention. For example, it has been held by the UK courts that trials conducted to discover something unknown or to test a hypothesis, to find out whether something which is known to work in specific conditions would work in different conditions, or even perhaps to see whether the experimenter could manufacture commercially in accordance with the patent can be regarded as experiments and exempted from infringement. Researchers in any particular country who wish to obtain current information about their ability to conduct research projects without incurring patent infringement liability should contact the patent office or an attorney in their respective countries. Unfortunately, there is very little literature addressing these issues, and because the law is constantly changing, older articles may not provide accurate information.

Even with uncertainties, it is clear that in many locations, researchers conducting basic research without a commercial end are free to practice in their field without fear or concern about the patent rights of others. Researchers at corporations likely cannot take advantage of such an infringement exemption. For researchers involved in work with a commercial link (e.g., researchers at private corporations, diagnostic laboratories reporting patient results for fees, academic research laboratories with private corporate collaborations, and the like), a license may be required. Unfortunately, each case needs to be evaluated on its own facts to determine whether a license is required and no general formula can be given. However, many corporations have personnel responsible for analyzing the need for, and acquisition of, patent rights. As such, bench scientists can generally go about their work without the burden of worrying about patent rights, or at a minimum, need only know the basic principles and issues so as to inform the appropriate personnel if potential patent issues arise.

### **3.3. Not Every Patent Is a Valid Patent**

In addition to the experimental use exception, researchers, including commercial researchers, may obtain freedom from the early PCR patents because of problems with the patents themselves. Although issued patents are presumed valid and are enforceable until a court of law says otherwise, the early PCR patents have begun to fall under scrutiny and may not be upheld in the future such that the basic reagents and methods

are no longer covered by patents. It must be emphasized that at this time most of the patents are still deemed valid and enforceable. However, researchers may wish to follow the events as they unfold with respect to the enforceability and validity of the PCR patents.

The first blow against the PCR patents was struck by Promega Corporation (Promega; Promega Corporation is a corporation headquartered in Madison, Wisconsin that produces for sale reagents and other products for the life science community.). HoffmannLaRoche filed an action against Promega on October 27, 1992 alleging breach of a contract for the sale of *Taq* DNA Polymerase (*Taq*), infringement of certain patents—the PCR Patents (United States Patent Nos. 4,683,195 and 4,683,202) and United States Patent No. 4,889,818—and related causes of action. At issue was United States Patent No. 4,889,818 (the ‘818 patent), entitled “Purified Thermostable Enzyme.” Promega denied the allegations of the complaint and claimed, among other things, that the ‘818 patent was obtained by fraud and was therefore unenforceable. After a trial in 1999, a US court held that all of the claims of the ‘818 patent unenforceable for inequitable conduct or fraud. The unenforceable claims are provided below.

1. Purified thermostable *Thermus aquaticus* DNA polymerase that migrates on a denaturing polyacrylamide gel faster than phosphorylase B and more slowly than does bovine serum albumin and has an estimated molecular weight of 86,000 to 90,000 Dalton when compared with a phosphorylase B standard assigned a molecular weight of 92,500 Dalton.
2. The polymerase of claim 1 that is isolated from *Thermus aquaticus*.
3. The polymerase of claim 1 that is isolated from a recombinant organism transformed with a vector that codes for the expression of *Thermis aquaticus* DNA polymerase.

The court concluded that Promega had demonstrated by clear and convincing evidence that the applicants committed inequitable conduct by, among other things, withholding material information from the patent office; making misleading statements; making false claims; misrepresenting that experiments had been conducted when, in fact, they had not; and making deceptive, scientifically unwarranted comparisons. The court concluded that those misstatements or omissions were intentionally made to mislead the Patent Office. The court’s decision has been appealed, and a decision from the Federal Circuit Court of Appeals is expected shortly. Pending the appeal court decision, the ‘818 patent is unenforceable.

Patents have also been invalidated in Australia and Europe. On November 12, 1997, the Australian Patent Office invalidated all claims concerning native *Taq* DNA polymerase and DNA polymerases from any other *Thermus* species, contained in a patent held by Hoffmann-La Roche (application no. 632857). The Australian Patent Office concluded that the enzyme had been previously purified in Moscow and published by Kaledin et al. (6) and that certain patent claims were unfairly broad. Although the case has been appealed, as of this writing, the *Taq* patent in Australia is unenforceable.

In Europe, on May 30, 2001, the opposition division of the European Patent Office held that claims in the thermostable enzyme patent EP 0258017B1 (a patent equivalent to the ‘818 patent in the United States) were unpatentable because they lacked an inventive step in view of previous publications to Kaledin et al. (6) and Chient et al. (7), as well as knowledge generally known in the field at the time the patent application was filed.



Although it has not been determined yet whether the PCR method patents were procured with the same types of misleading and deceptive behavior, the PCR patents have been challenged based on an earlier invention by Dr. Gobind Khorana and coworkers in the late 1960s and early 1970s. Under US and many international patent laws, patent claims are not valid if they describe an invention that was used and/or disclosed by others prior to the filing date of the patent. The principle behind such rules is to prevent people from patenting, and thus removing from the public domain, things that the public already owns. Although the PCR patents make no mention of such work, DNA amplification and cycling reactions were conducted many years before the filing of the PCR patents in the laboratory of Dr. Khorana. Dr. Khorana's method, which he called "repair replication," involved the steps of the following: (1) extension from a primer annealed to a template; (2) separating strands; and (3) reannealing of primers to template to repeat the cycle. Dr. Khorana did not patent this work. Instead he dedicated it to the public. Unfortunately, at the time that Dr. Khorana discovered his amplification process, it was not practical to use the method for nucleic acid amplification, and the technique did not take off as a commercial method. At the time this work was disclosed, chemically synthesized DNA for use as primers was extremely expensive and cost-prohibitive for even limited use. Additionally, recombinantly produced enzymes were not available. Thus, not until the 1980s, when enzyme and oligonucleotide production became more routine, could one economically replicate Dr. Khorana's methods.

The validity of the PCR patents was challenged in 1990 by E.I. Dupont De Nemours & Co. (Dupont). Based on publicly available records, it appears that Dupont pointed to the work from Dr. Khorana's laboratory, arguing that all of the method steps required in the basic PCR method were taught by Dr. Khorana's publications and were in fact in the public domain. Hoffmann-LaRoche (who was positioned to acquire the technology) out-maneuvered Dupont by putting the Khorana papers in front of the United States Patent and Trademark Office in a reexamination procedure. Under reexamination, the patent holder has the ability to argue the patentability of an invention to the patent office without any input allowed by third parties, such as Dupont. As shown by publicly available records, during the reexamination procedure expert declarations were entered to raise doubt about the teaching of the Khorana references. As a result (not surprisingly), the Patent Office upheld the patents. Once a patent has issued in view of a reference, there is a strong presumption of validity that courts must acknowledge in any proceedings that later attempt to invalidate the patent in view of the reference.

In addition to the disadvantage caused by the reexamination procedure, publicly available records show that Dupont was not able to use several pieces of compelling evidence against the PCR patents. Dupont, although performing clever replication work to show the sufficiency of Dr. Khorana's disclosures (in direct contrast to the expert declarations submitted to the Patent Office during reexamination), did not submit the data in a timely manner in the proceedings. The judge ruled that the data should be excluded as untimely and prejudicial. Dupont also found additional references disclosing the earlier invention by Khorana, but did not provide them to the court in time and they were not considered. Thus, it seems that validity of the PCR patents was never truly tested in view of the work conducted by Dr. Khorana and his colleagues. Such a test, as well as others, may come in the near future as part of the Promega/HoffmannLaRoche litigation.

Should these or any additional patents be found invalid and unenforceable, the patent issues for researchers wishing to practice PCR will be greatly simplified. Interestingly, if it is found that one or more of the invalid or unenforceable patents were used to suppress competition in the market or to unfairly control the freedom of researchers, companies exerting such unfair market control may be subject to laws designed to prevent unfair and anticompetitive behavior. If a court were to rule that anticompetitive behavior was exercised, the violating patent owner may be forced to compensate those that were harmed. Although it is impossible to predict at this time the outcome of future court proceedings, researchers may wish to follow the progress of these cases. At a minimum, they offer perspective into the patent world and provide important subject matter for debate that is extremely relevant to shaping the future of patent public policy, an area that will increasingly play a role in the day-to-day lives of scientists.

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