CONFIDENTIAL - SUBJECT TO PROTECTIVE ORDER

IN THE COURT OF CHANCERY OF THE STATE OF DELAWARE

IN AND FOR NEW CASTLE COUNTY

EASTMAN KODAK COMPANY, Plaintiff, v. CETUS CORPORATION, Defendant, and CHIRON CORPORATION, Intervenor.	Civil Action No. 12249

AFFIDAVIT OF JOHN J. SNINSKY, Ph.D.

I, John J. Sninsky, being first duly sworn, do depose and say:

1. I have spent my professional life working in the area of molecular biology and virology. I received a Ph.D. in biology from Purdue University in 1976 and worked as a postdoctoral fellow at the Stanford University Department of Medicine from 1976-1980. I then spent 1981-1984 as an Assistant Professor at Albert Einstein College of Medicine in the Department of Microbiology and Immunology, with a joint appointment in the Department of Molecular Biology. I joined Cetus as Senior Scientist in the Department of Microbial Genetics, and became Senior Director of Diagnostics of the PCR Division in 1988. At Cetus, I have directed the company's efforts in research on the detection of viruses and other pathogens. I have authored over 50 scientific publications

ATTORNEY'S EYES ONLY
RESTRICTED

Sninsky
EXHIBIT NO. 350
DATE 7/27/06
GINA GLANTZ - NO. 9795

RMS 71061

relating to genetic engineering and virology. My laboratory was the first to use PCR technology in the detection of the HIV virus, and published the first paper on that subject. I have lectured extensively on the use of PCR in diagnostics, and am co-editor with Drs. T. White, M. Innis and D. Gelfand of the book PCR Protocols published in 1990 by Academic Press. Further details are set forth in my Curriculum Vitae, which is attached hereto as Exhibit A.

2. The polymerase chain reaction ("PCR") was invented by Dr. Kary Mullis, a Cetus scientist, in 1983. Over the next two years Dr. Mullis and several other Cetus scientists, including Henry Erlich and Norman Arnheim, worked on improving and using this process, and by late 1985 published a detailed description of their work involving the amplification and detection of DNA. The patent directed to the basic PCR amplification process is United States Patent No. 4,683,202 (the "202 patent") and the patent directed to the use of the process as a detection method is United States Patent No. 4,683,195 (the "195 patent"). Cetus owns both of these patents. All of the scientific work required in these patents was performed well before February 1986.

3. PCR is a technique for selectively amplifying a particular nucleic acid sequence or gene segment that is present in a sample in extremely small quantities. PCR thus allows scientists to detect information occurring in DNA or RNA that would otherwise be very difficult or impossible to detect. This is sometimes analogized to converting a needle in a haystack to a haystack of needles.

The PCR process is a cyclical method, each cycle of which consists of three steps. The first step is known as "denaturation" and is the separation of the two strands of DNA. DNA is a very long molecule described as a double-stranded helix. Each strand consists of a series of building blocks or components, also known as "nucleotides." The bases of each nucleotide along one of the strands are bound or match to the bases along the other strand in a complementary fashion. The base known as Guanine always binds with the base known as Cytosine, and the base known as Adenine always binds with the based known as Thymidine. During denaturation, the two strands are separated, usually by heating the DNA to very high temperatures. If one wishes to amplify a region of RNA, this RNA is first converted to a complementary DNA (cDNA).

The second step of the PCR process is known as "annealing" or binding. During this step, typically two short synthetic pieces of DNA (or primers) made up of nucleotides bind to the regions of the two complementary strands of the sample DNA that they match. The primers must be designed so that they will target only the sequence that you want to copy.

The third step in PCR is known as "extension." During this step, an enzyme, known generically as a DNA polymerase, makes new DNA in a template-dependent manner by adding nucleotides to one end of each primer to fill out a new strand that is the precise complement of the original strand. This extension must be done in a way such that the new complementary strand (the "extension product") can and does serve as a template for another cycle of denaturation, annealing and extension.

Thus, using the PCR process, more and more copies of specific segments of DNA can be made, using the products of one cycle as templates to make more products in later cycles.

This process increases the amount of new DNA in a dramatic, exponential way. The cycle can be repeated numerous times, resulting in a million to a billion-fold increase in the amount of the target DNA sequence contained in the sample. Attached to this Affidavit as Exhibit B is a schematic showing the steps of the PCR reaction.

4. PCR can be applied in many scientific fields and its invention has revolutionized all of science. Scientific fields in which PCR has application include human, animal and plant diagnostics, forensics, anthropology, environmental monitoring, identity testing, tissue typing, biological research, and agricultural genetic engineering, to name but a few. Among the most important currently known uses are research, human diagnostics, forensics, identity testing and environmental uses.

"Research" generally refers to the use of PCR in laboratories to increase scientific knowledge and understanding about DNA. In this context, PCR can be used to study DNA or RNA generally, or specific segments of DNA associated with, for example, a disease or production of a particular protein. This is the type of work carried out by Dr. Rick Myers of UCSF as well as thousands of other scientists throughout the world. I understand that Dr. Myers will describe this work in greater detail in his Affidavit. In addition, PCR could be used to study the effectiveness of new, experimental therapeutics.

"Human diagnostics" generally refers to the detection, in a clinical sample, of a target sequence indicative of a particular disease, condition, or genetic trait relevant to a medical condition. Diagnosis generally can be performed "in vivo" -- that is, on the living human body -- or "in vitro" -- that is, on a sample taken from the human body and placed in a test tube or other container. Generally, PCR is used only in in vitro tests. In the commercial setting, the diagnostic test may be performed by the physician, his or her staff or hospital laboratory by purchasing a commercially available diagnostic product, or the physician may send a tissue sample taken from the patient to a reference laboratory. A reference laboratory is an institution that offers the service of performing specified tests on samples sent in by physicians or hospitals.

"Forensics" generally refers to the science of criminal investigation and detection. DNA from hair, skin, blood or other human tissue found at the scene of a crime can be compared to the DNA of a suspect or suspects. Crime investigators can thereby conclusively determine whether or not the suspect or suspects were at the scene of the crime.

The "identity testing" field includes the use of PCR to determine parentage, and the use of PCR to identify, for example, military personnel killed or missing in action.

PCR can also be used in the environmental field. An example of this use is the use of PCR to determine whether legionella -- the bacteria that causes legionnaire's disease -- is present in buildings. Another example is the use of PCR to

test a water supply for the presence of bacteria dangerous to humans.

5. For all of these fields, the same PCR technology described in Cetus' patents is used. The difference is in the objective, not in the technology.

6. I was generally aware of the negotiations between Cetus and Kodak in 1985 and the beginning of 1986. I gave presentations to Kodak about Cetus' technology as a part of those negotiations. In addition, I served as a member of the management committee for the Cetus-Kodak Research And Development Program ("R&D Program") for all three years of the R&D Program -- that is, from February 1986 until February 1989. The Program's management committee was known as the "Research And Development Program Management Council," or the "RDMC." I am therefore very familiar with the R&D Program's activities and objectives.

7. The Cetus Kodak R&D Program focused on research and the development of in vitro human diagnostic products for commercial markets. In particular, while in theory the Program could have developed products based on several different technologies, in practice the Program focused on products based on monoclonal and polyclonal antibody technology. During the discussions between Cetus and Kodak scientists prior to the signing of the 1986 Agreement, Kodak scientists were clearly more interested in Cetus' technology relating to monoclonal antibodies than in PCR technology. In 1986, at the outset of the Program, development of monoclonal antibody technology had already reached a fairly advanced stage. The Kodak members of the RDMC, who controlled the focus of the Program, were consistently and from

the outset concerned with developing products that would have a rapid return on investment due to directives by Kodak upper management. In RDMC meetings, the Kodak RDMC members constantly reiterated Kodak's position that the R&D Program had to yield short term financial success. In RDMC meetings, the Kodak members of the RDMC insisted that a focus on antibody products was necessary to achieve Kodak's objective of a rapid return on investment. They also insisted that the Program must achieve this rapid return on investment before Kodak would devote the required resources to developing PCR products.

8. The parties engaged in both research and product development work under the R&D Program. The focus of the RDMC and the R&D program was, however, on developing specific products. What we meant by a "product" in the RDMC was a kit consisting of the biological reagents (such as monoclonal antibodies) and other materials necessary to perform a test for the detection of a particular disease or medical condition by the targeted customer. Such kits would have to be reliably manufactured under rigid manufacturing protocols. Under the R&D Program we developed such kits based on antibody technology for Strep A, Chlamydia, hCG (a pregnancy test) and Herpes Simplex Virus.

Concurrently, the joint venture between Cetus and Perkin Elmer known as PECI (for "Perkin Elmer-Cetus Instruments") developed PCR-based kits, instruments and reagents for use in various research applications. A catalogue for these products is attached to this Affidavit as Exhibit C. These products are used in conjunction with an instrument developed by PECI for use in

PCR testing known as a thermal cycler. The purpose of the instrument is to carry out temperature cycles in precise, programmed sequences so that PCR amplification will occur. Catalogues for the PECI instrument are attached hereto as Exhibit D.

9. The RDMC established timelines and budgets for feasibility projects. After the RDMC decided to consider a potential feasibility study for a target disease or condition, Cetus would engage in research to gain more information and determine whether it was possible to accomplish technically. This was a substantial effort, and included designing a series of reagents and procedures to detect a pathogen or target sequence. This research work included demonstrating that the information gathered had utility over already available products in the commercial arena. Specifically, research was required for locating and analyzing a relevant portion of the target DNA sequence and designing primers and probes specific to that sequence. For example, the HIV virus can take many different forms. We had to determine what nucleic acid sequences were common to all variants in order to provide directed amplification. We also had to develop appropriate and often complicated sample preparation procedures and a possible detection format to accommodate all variants. All of this helps to demonstrate why merely designating a target for which you would like to have a product is a long way from actually beginning product development. To my knowledge, the only PCR-based project on which product development began during the R&D Program was an HLA DQ Alpha test kit.

10. During the term of the Program, the RDMC did not use the term "product" for the format for an assay. For example, the antibody products developed under the program were all assays utilizing a format known as the "SureCell" format. The SureCell format was not listed as a product in the R&D Program's Milestone Reports; the particular test kits developed using the format were listed as products. Illustrative Milestone Reports are attached to this Affidavit as Exhibit E. As soon as the RDMC determined that both technical and commercial feasibility had been demonstrated, the project was included on the RDMC's list of current products, although substantial development work had still to be done before a marketable product would be available. The RDMC did not consider anything not included on the list of current products to be a product under development. No PCR product was so designated as a current product. Attached hereto as Exhibit F is the milestone report dated August 19, 1988, which was the last milestone report of the Cetus Kodak R&D Program. All of the "current" products listed on the milestone report are antibody products.

11. Cetus and Kodak successfully developed to completion three antibody-based products under the Program. The Herpes Simplex Virus test kit was FDA approved following the R&D collaboration. There were, however, no PCR-based products developed under the Program. In addition, there was only one PCR project that had reached the product development stage. This was a test kit for DQ alpha. DQ alpha is one of the HLA genes which are associated with human identity. Therefore, DQ alpha was not considered a diagnostic target of the R&D Program. Cetus,

however, wanted to overcome Kodak's prejudice against developing PCR-based products, and therefore offered to Kodak the DQ alpha test kit, which was then at an advanced stage of development for forensic use, as a possible kit to test the compatibility of organ transplants. Eventually, Cetus completed a DQ alpha test kit although the test kit Cetus completed for forensic use was different from the one worked on under the Program. A picture of the Cetus DQ alpha test kit is attached hereto as Exhibit G.

12. During the term of the Program, Kodak was working on an instrument Kodak called an "Ektamizer" and a container for use on that instrument which Kodak called an "Ektapod". The Ektamizer and Ektapod were for sample preparation, amplification and detection. The pod, which was designed for use on the Ektamizer, was a rigid plastic container with a metal foil bottom in which reagents and samples would eventually be placed. To the best of my knowledge, these are the only instruments, devices, or containers that Kodak worked on during the Program that may be relevant to PCR.

13. The Ektamizer and Ektapod approach which Kodak worked on under the R&D Program never worked. One of the insurmountable problems arose from the fact that the friction between the Ektapod and the Ektamizer stations (e.g. temperature plates) caused the clutch to repeatedly burn out. This necessitated that studies using the pods be carried out by manually moving them from one hot plate to another. I recall RDMC meetings in late 1987 and early 1988 in which we discussed whether it would be possible to recover any "salvage value" from the Ektamizer. Attached hereto as Exhibit H is a copy of the

Kodak Report of the December 7, RDMC Meetings in which this topic was discussed.

14. From my personal conversations with Kodak employees, I know that Kodak was aware of the PECI joint venture between Cetus and Perkin Elmer, which Cetus entered into in December, 1985 and announced publicly. The activities of PECI included the development and commercial sale of an instrument for use with PCR-based tests and the research and development of PCR-based test kits for research purposes. As I mentioned earlier in this Affidavit, PECI did develop and now sells PCR test kits for use in various research applications, including the analysis of HIV and reagents for the analysis of other viruses and genes. PECI catalogues showing available test kits and thermal cyclers are attached as Exhibit C and D to this Affidavit.

15. During the R&D Program, Cetus regularly provided Kodak with copies of reports prepared for internal distribution at Cetus concerning progress on research and development in diagnostics and technologies that might be relevant to diagnostics. Cetus provided substantially more information to Kodak than it received from Kodak relating to Kodak's own activities. Cetus also kept Kodak scientists informed regarding generally applicable research at Cetus that might be useful for diagnostics. Furthermore, Cetus attempted to teach Kodak scientists about the general properties of PCR, and reagents useful in performing PCR.

16. By the end of 1987 Cetus was extremely frustrated by Kodak's refusal to devote the necessary resources to PCR. In a special RDMC meeting in December, 1987 the Cetus members of the

RDMC emphasized to Kodak the need for Kodak to devote more resources to PCR. A copy of the Kodak report of the December 7, 1987 RDMC meeting is attached hereto as Exhibit I. During the winter of 1988 we attempted to convince Kodak to devote more resources to the research and development of PCR. Kodak resisted these efforts, so Cetus began to look for other companies that might be interested in developing PCR with Cetus. Kodak would not, however, agree to a three-way collaboration. Consequently, in February, 1989 the Cetus-Kodak Program expired and Cetus entered into a collaboration and license agreement with Hoffmann-La Roche, Inc. and F. Hoffmann-La Roche, Ltd. (collectively "Roche") involving the use of PCR in diagnostic products and services.

17. I have worked with Roche on the Cetus-Roche collaboration since it began in February, 1989. The Cetus-Roche collaboration is solely devoted to research and development on PCR diagnostics. The Cetus-Roche collaboration, however, like the Cetus-Kodak R&D Program, is directed to in vitro human diagnostics, although the Cetus-Roche collaboration is broader as it includes services as well as products. In complete contrast to Kodak, Roche has demonstrated a high level of commitment and has devoted substantial resources including funding, personnel and facilities, to the development of PCR. Although Roche has not yet begun marketing a PCR-based product, Roche has developed a number of PCR-based test services that are currently being offered by Roche Biomedical Labs.

In addition to the work funded by Roche, Cetus has continued to work on PCR research with its own funds. Since

February, 1989, Cetus has spent approximately $20 million on PCR research and development.

18. I am not aware of any significant development on PCR generated in the course of the Cetus-Kodak R&D Program that has not been publicly disclosed. Cetus has an aggressive intellectual property review allowing rapid publication dating from before the Cetus Kodak Program. This publication policy is important to Cetus' ability to attract scientists who are leaders in their fields. Scientists' accomplishments are measured in large part by the number and quality of their publications. Therefore, a company like Cetus whose charter it is to attract the best scientists available must have a rapid publication review policy without precluding possible patent rights.

Under Cetus' publication review policy, all discoveries by a scientist must be submitted to an in-house patent committee for consideration. If it is determined by the patent committee that the discovery is patentable, then a scientist may submit his or her manuscript describing the discovery such that publication would not occur prior to the appropriate patent application. Cetus attempts to file patent applications as quickly as possible so that a scientist is not delayed in being able to publish a paper concerning his or her discovery so that valuable information can be relayed to his or her colleagues in the scientific community. If the patent committee determines not to file for a patent, the scientist is immediately free to publish his or her discovery in a scientific journal or otherwise. The time between a scientist's discovery and his or her ability to publish generally ranges from one month to one year.

Cetus' publication policy is typical of publication policies in the biotechnology industry. Specifically with respect to PCR, there have been at least 7,000 publications concerning PCR since the first paper in December of 1985. Attached to the Affidavit of George McGregor is a bibliography of PCR publications demonstrating the extensive knowledge regarding PCR that is publicly available. Because of this rapid publication review policy, and because of the pace of development in the biotechnology industry, all Cetus technology of any value relating to PCR discovered as of the beginning of 1989 is in the public domain or is the subject of an issued patent. Consequently, if there is anything secret remaining from the 1986 to 1989 time period, it is not of utility today.

19. Attached hereto as Exhibit I is a copy of a book or manual entitled PCR Protocols published in 1990. The editors of this manual include myself and other Cetus scientists and Dr. Thomas White, a former Cetus scientist who joined Roche in March, 1989.

20. The information necessary to use PCR to detect genetic or viral material associated with particular diseases or medical conditions is also publicly available through the PCR-based test kits sold by PECI for research purposes. PECI has developed and sells, for example, primer and probe sets for use in detecting and analyzing HIV. Attached hereto as Exhibit J are some sample package inserts for these products. Although these kits are not sold for diagnostic uses, their product inserts and the publications listed in them provide a more complete and advanced description of the use of PCR to analyze HIV than the

information generated between 1986 and 1989. Because of the rapid advances in HIV research using PCR, it is inconceivable that researchers would benefit from outdated, unpublished data prior to February 1989. Even failed experiments and unsuccessful approaches, which are generally not published in the scientific literature, are readily discussed at scientific conferences, if not implicit in the published literature. Therefore, I do not believe that any "lead time" would be associated with a body of knowledge generated before February, 1989.

21. As I explained earlier in this Affidavit, PCR is a technique with applications throughout the life sciences. PCR is not a technology peculiar to diagnostic products. To the contrary, there is no particular piece of technology that is used in in vitro human diagnostic products that is not used in some other field. For example, DNA Taq polymerase, of which I am aware Kodak claims exclusive rights of some kind, is a DNA polymerase enzyme that can be used in every PCR application. The use in PCR of thermostable enzymes, a group in which Taq DNA polymerase is included, is in fact covered by Cetus' earliest PCR patents for which applications were filed prior to any work on the R&D Program, as well as Cetus U.S. Patent No. 4,965,188. Therefore, the use of Taq DNA polymerase in PCR is widely known in the scientific community.

22. If the Cetus-Roche PCR sale does not close, there is a serious question of Cetus' financial ability to continue the Roche-Cetus collaboration and Cetus' other PCR work. The uncertainty created by blocking the Roche-Cetus transaction would make it difficult for Cetus to retain its own scientific staff

and in addition could threaten Cetus' relationship with its collaborating researchers and investigators. Moreover, if Cetus' financial problems threaten its funding of the PECI program, then necessary reagents and instruments being utilized both in research and commercially may become unavailable, or their supply restricted. If the availability of these products is interrupted, experiments and research will be delayed or in some instances scrapped, since a change in reagent or instrument could render data gathered using other such products unusable. In addition, the uncertainty surrounding the blocking of the Roche transaction could very well delay the introduction of valuable, beneficial diagnostic products. For example, Roche has announced that an HIV-1 test is in clinical trials and may be presented to the FDA in early 1992. This product utilizes PECI products. As noted above, the financial harm to Cetus that would result from an injunction threatens the supply of these PECI products to Roche. Even if the PECI supply is somehow protected, uncertainty created by the injunction and the potential disruption of funds and reagents to support collaborators, all threaten to delay the development and introduction of PCR products.

23. When the harm to the public from any injunction that would slow the advancement of research in PCR technology is considered, there is yet a further equity of which, in my opinion, the Court should take cognizance, and that is this: During the three year collaboration from February, 1986 through February, 1989, Kodak essentially starved the development of PCR technology. Kodak spent the majority of the funds available during the Kodak-Cetus R&D program on other technology, not on

PCR. Even as to the funds that Kodak did spend in the area of PCR, its preoccupation was never on the development of the technology, but rather on the development of a mechanical apparatus, which they referred to as an Ektamizer. Suitable mechanical devices existed at the time and exist now for performing the PCR process, and these are available commercially for a minimal cost -- less than $15,000. For reasons of its own, Kodak ignored this fact. As a result of Kodak's attitude, no PCR diagnostic products were advanced to the stage that even merited discussion with the FDA, a necessary prerequisite for availability to the public during the period February 4, 1986 through February 4, 1989. Then, for the almost three years that have followed the end of the Kodak-Cetus R&D program, Kodak has relayed little, if any, information to the scientific community as a whole in the PCR area. It appears to me that they have devoted limited resources to that effort. Kodak has produced no PCR assays, tests, products, or services of any kind that can be used by or for the public. Kodak has published relatively little on the subject. During the same period, Cetus and Roche have made substantial advances in this area and have made publicly available PCR diagnostic services for such serious human conditions as the Human Immunodeficiency Virus (the causative agent in AIDS), Cystic Fibrosis, Sickle Cell Anemia, and several others including Human Papillomavirus, a possible precursor of cervical cancer. As a result of these advances, people's lives have been lengthened and it is quite probable that people's lives have been saved by, for example, early detection and confirmation of the presence of HIV, thereby giving those individuals the

information they needed to prevent the spread of that infection to others. I can say without a doubt that had the development of PCR technology been left to Kodak, none of these important advances would have occurred. Now, instead of accelerating its own program, Kodak's tactic is instead to attempt to throw whatever roadblocks it can in the way of those who are advancing this important area of science. As one who participated in the Cetus-Kodak R&D program personally for three years, I can say categorically that is was never my understanding that Kodak had "exclusive" rights to use PCR in human diagnostics, nor did any of the Kodak representatives ever make such a claim during the program. I can also say that Kodak's current claim to "exclusive" rights in the area of PCR technology is not in the public interest; indeed, it is directly contrary to the public interest. The public interest will be served by having more financial and human resources devoted to the advancement of PCR research. Unfortunately, due to its financial condition, Cetus cannot do so. Kodak has demonstrated that it can not or will not do so. Based upon my association with the Cetus-Roche Program during the past three years, my opinion is that Roche has both the ability and commitment to advance PCR research broadly and to ensure the availability of PCR testing, as rapidly as possible, to the general public.

John J. Sninsky

SWORN TO BEFORE ME and subscribed in my presence this 14th day of November, 1991.

Notary Public

EXHIBIT A

CURRICULUM VITAE

NAME: John J. Sninsky, Ph.D.

DATE OF BIRTH: March 12, 1950

CURRENT POSITION: Senior Director, Diagnostics Research

EDUCATION:

1972	Bates College / B.S. Lewiston, Maine
1976	Purdue University / Ph.D. West Lafayette, Indiana

POST-GRADUATE TRAINING:

1976-1980	Postdoctoral Fellow Departments of Genetics and Medicine Stanford University School of Medicine Stanford, California

PROFESSIONAL EMPLOYMENT:

1981-1984	Assistant Professor Department of Microbiology & Immunology Albert Einstein College of Medicine
1981-1984	Assistant Professor Department of Molecular Biology Albert Einstein College of Medicine
1984-present	Visiting Professor Department of Microbiology & Immunology Albert Einstein College of Medicine
1984-1985	Senior Scientist Department Microbial Genetics Cetus Corporation
1985-1988	Director, Diagnostics Program Director, Senior Scientist Department of Infectious Diseases Cetus Corporation
1988-	Senior Director, Diagnostics Program PCR Division Director, Senior Scientist Department of Infectious Diseases Cetus Corporation

PROFESSIONAL SOCIETY MEMBERSHIP:

American Society for Microbiology
American Association for the Advancement of Science

AWARDS AND HONORS:

1972	Honors in Biology, Bates College
1973-1976	N.I.H. Traineeship Research
1977	Dean's Fellowship, Stanford University
1977-1979	American Cancer Society Postdoctoral Fellowship
1979-1980	Dean's Fellowship, Stanford University

PUBLICATIONS:

Sninsky, J.J., Bennett, G.N., and Gilham, P.T. (1974) "Single-Addition and transnucleotidation reactions catalyzed by polynucleotide phosphorylase. Effect of enzymatic removal of inorganic phosphate during reaction." *Nucl. Acids Res.* 1:1655-1674.

Sninsky, J.J., Last, J.A., and Gilham, P.T. (1976) "The use of terminal blocking groups for the specific joining of oligonucleotides in RNA ligase reactions containing equimolar concentrations of acceptor and donor molecules." *Nucl. Acids Res.* 3:3157-3166.

Hawley, D.M., Sninsky, J.J., Bennett, G.N., and Gilham, P.T. (1978) "Activity of polynucleotide phosphorylase with nucleoside diphosphates containing sugar ring modification." *Biochemistry* 17:2082-2086.

Sninsky, J.J., Siddiqui, A., Robinson, W.S., and Cohen, S.N. (1979) "Cloning and endonuclease mapping of the hepatitis B viral genome." *Nature* 279:346-348

Sninsky, J.J., Uhlin, B.E., Gustafsson, P., and Cohen, S.N. (1981) "Construction and characterization of a novel two-plasmid system for accomplishing temperature-regulated, amplified expression of cloned adventitious genes in *Escherichia coli*." *Gene* 16:275-286.

Sninsky, J.J. and Cohen, S.N. (1982) "Specialized cloning vectors for hepatitis B virus gene expression in *Escherichia coli*." *Hepatology* 2:72S-78S.

Chase, J.W., Murphy, J.B., Whittier, R.F., Lorensen, E., and Sninsky, J.J. (1983) "Amplification of *ssb-1* mutant single-stranded DNA-binding protein in *Escherichia coli*." *J. Molec. Bio.* 164:193-211.

Schottel, J.L., Sninsky, J.J., and Cohen, S.N. (1984) "Effects of alterations in the translation control region on bacterial gene expression: Use of *cat* gene constructs transcribed from *lac* promoter as a model system." *Gene* 28:177-193.

PUBLICATIONS: (cont.)

Schaeffer, E., and Sninsky, J.J. (1984) "Predicted secondary structure similarity in the absence of primary amino acid sequence homology: hepatitis B virus open reading frames." *Proc. Natl. Acad. Sci. USA* 81:2902-2906.

Friefeld, B., Korn, R., DeJong, P., Sninsky, J.J., and Horwitz, M. (1985) "The 140-kDa adenovirus DNA polymerase is recognized by antibodies to *Escherichia coli*-synthesized determinants predicted from an open reading frame on the adenovirus genome" *Proc. Natl. Acad. Sci. USA* 82:2652-2656.

Wong, D.T., Nath, N., and Sninsky, J.J. (1985) "Identification of hepatitis B virus polypeptides encoded by the entire pre-s open reading frame." *J. Virol.* 55:223-231.

Meyers, M.L., Vitvitskyi-Trepo, L., Nath, N., and Sninsky, J.J. (1986) "Hepatitis B virus polypeptide X: Expression in *E. coli* and identification of specific antibodies in sera from HBV-infected humans." *J. Virol.* 57:101-109.

Schaeffer, E., Snyder, R., and Sninsky, J.J. (1986) "Identification and localization of pre-s-encoded polypeptides from woodchuck and ground squirrel hepatitis viruses." *J. Virol.* 57:173-182.

Rodgers, G.C., Laird, W.J., Coates, S.R., Mack, D.H., Huston, M., and Sninsky, J.J. (1986) "Serological characterization and gene localization of an *Escherichia coli* expressed 37-kilodalton *Trepomena pallidum* antigen." *Infec. & Immun.* 53:16-25.

Pugh, J.C., Sninsky, J.J., Summers, J.W., and Schaeffer, E. (1987) "Characterization of a pre-S polypeptide on the surfaces of infectious avain hepadnavirus particles." *J. Virol.* 61:1384-1390.

Kwok, S., Mack, D.H., Mullis, K.B., Poiesz, B., Ehrlich, G., Blair, D., Friedman-Kien, A., and Sninsky, J.J. (1987) "Identification of human immunodeficiency virus sequences by using *in vitro* enzymatic amplification and oligomer cleavage detection." *J. Virol.* 61:1690-1694.

Ou, C.-Y., Kwok, S., Mitchell, S.W., Mack, D. H., Sninsky, J.J., Krebs, J. W., Feorino, P., Warfield, D., and Schochetman, G. (1988) "DNA amplification for direct detection of HIV-I in DNA peripheral blood mononuclear cells." *Science* 239:295-297.

Byrne, B., Li, J.J., Sninsky, J.J., and Poiesz, B. (1988) "Detection of HIV-1 RNA sequences by *in vitro* DNA amplification." *Nucl. Acids Res.* 16:4165.

Duggan, D.B., Ehrlich, G. D., Davey, F. P., Kwok, S., Sninsky, J.J., Goldberg, J., Baltrucki, L. and Poiesz, B.J. (1988) "HTLV-I induced lymphoma mimicking Hodgkin's disease diagnosis by polymerase chain reaction amplification of specific HTLV-I sequences in tumor DNA." *Blood* 71:1027-1032.

Bhagavati, S., Kula, R., Ehrlich, G., Kwok, S., Sninsky, J.J., Udami, V., and Poiesz, B. (1988) "Detection of human T cell lymphoma/leukemia virus-type I (HTLV-I) in the spinal fluid and blood of cases of chronic progressive myelopathy and clinical, radiological and electrophysiological profile of HTLV-I associated myelopathy." *N. Eng. Jour. Med.* 318:1141-1147.

PUBLICATIONS: (cont.)

Farzadegan, H., Polis, M.A., Wolinsky, S. M., Rinaldo, C. R., Sninsky, J. J., Kwok, S., Griffith, R. L., Kaslow, R. A., Phair, J. P., Polk, B. F., and Saah, A. J. (1988) "Loss of human immunodeficiency virus type I (HIV-1) antibodies with evidence of viral infection in asymptomatic homosexual men, a report from the Multicenter AIDS Cohort Study (MACS)." *Ann. Int. Med.* **108**:785-790.

Mack, D. H., and Sninsky J.J. (1988) "A sensitive method for the identification of uncharacterized members of known virus groups: Hepadnavirus model system." *Proc. Natl. Acad. Sci. USA* **85**:6977-6981.

Kwok, S., Ehrlich, G., Poiesz, B., Kalish, R., and Sninsky J. J. (1988) "Enzymatic amplification of HTLV-I viral sequences from peripheral blood mononuclear cells and infected tissues." *Blood* **72**:1117-1123.

Mack, D., Bloch, W. Nath, N., and Sninsky, J.J. (1988) "Hepatitis B virus particles contain a polypeptide encoded by the largest open reading frame: A putative reverse transcriptase." *J. Virol.* **62**:4786-4790.

Larzul, D., Guigue, F., Sninsky, J.J., Mack, D.H., Brechot, C., and Guesdon, J.-L. (1988) "Detection of hepatitis B virus sequences in serum by using in vitro enzymatic amplification." *J. Virol. Meth.* **20**:227-237.

Hart, C., Spira, T., Moore, J. Sninsky, J.J., Schochetman, G., Lifson, A., Galphin, J., and Ou, C.-Y. (1988) "Direct detection of HIV RNA expression in seropositive subjects." *Lancet* 596-599.

Rayfield, M., DeCock, K., Heyward, W., Goldstein, L., Krebs., J, Kwok, S., Lee, S., McCormick, J., Moreau, J.M., Odehouri, K., Schochetman, G., Sninsky, J.J., and Ou, C.-Y. (1988) "Mixed human immunodeficiency virus (HIV) infection in an individual: demonstration of both HIV type 1 and type 2 proviral sequences by using polymerase chain reaction." *J. Infec. Dis.* **158**:1170-1176.

Kwok, S., Kellogg, D., Erlich, G., Poiesz, B., Bhagavati, S., and Sninsky, J.J. (1988) "Characterization of a sequence of human T cell leukemia virus type I from a patient with chronic progressive myelopathy." *J. Infec. Dis.* **158**:1193-1197.

Abbott, M.A., Poiesz, B.J., Byrne, B.C., Kwok, S., Sninsky, J.J., and Erlich, G.D. (1988) "Enzymatic gene amplification: qualitative and quantitative methods for detecting proviral DNA amplified in vitro." *J. Infec. Dis.* **158**:1158-1169.

Thiers, V., Kremsdorf, D., Schellekens, H., Goudeau, A., Sninsky, J.J., Nakajima, E., Mack, D., Driss, F., Wands, J., Tiollais, P., and Brechot, C. (1988) "Transmission of hepatitis B from hepatitis-B-seronegative subjects." *Lancet* 1273-1276.

Imagawa, D.T., Lee, M.H., Wolinsky, S.M., Sano, K., Morales, F., Kwok, S., Sninsky, J.J., Nishanian, P.G., Giorgi, J., Fahey, J.L., Dudley, J., and Visscher, B.R. (1989) "Human immunodeficiency virus type 1 infection in homosexual men who remain seronegative for prolonged periods." *N. Eng. Jour. Med.* 320:1458-1462.

PUBLICATIONS: (cont.)

Ulrich, P.P., Ramesh, A.B., Seto, B., Mack, D., Sninsky, J.J., and Vyas, G.N. (1989) "Enzymatic amplification of hepatitis B virus DNA in serum compared with infectivity testing in chimpanzees." *J. Infec. Dis.* **160**:37-43.

Meyerhans, A., Cheynier, R., Albert, J., Seth, M., Kwok, S., Sninsky, J.J., Morfeldt-Manson, L., Asjö, B., and Wain-Hobson, S. (1989) "Temporal fluctuations in HIV quasispecies in vivo are not reflected by sequential HIV isolations." *Cell* 58:901-910.

Goodenow, M., Huet, T., Saurin, W., Kwok, S., Sninsky, J.J., and Wain-Hobson, S. (1989) "HIV-1 isolates are rapidly evolving quasispecies: evidence for viral mixtures and preferred nucleotide substitutions." *J. Acquired Immune Def. Syndromes* 2:344-352.

Jackson, J.B., Kwok, S.Y., Hopsicker, J.S., Sannerud, K.J., Sninsky, J.J., Edson, J.R., and Balfour,Jr., H.H. (1989) "Absence of HIV-1 infection in antibody-negative sexual partners of HIV-1 infected hemophiliacs." *Transfusion* 29:265-267.

Shibata, D., Bymes, R.K., Nathwani, B., Kwok, S., Sninsky, J.J., and Arnheim, N. (1989) "Human immunodeficiency viral DNA is readily found in lymph node biopsies from seropositive individuals. Analysis of fixed tissue using the polymerase chain reaction." *Am. J. Pathol.* 135:697-702.

Chadwick, E.G., Yogev, R., Kwok, S., Sninsky, J.J., Kellogg, D.E., and Wolinsky, S.M. (1989) "Enzymatic amplification of the human immunodeficiency virus in peripheral blood mononuclear cells from pediatric patients." *J. Infec. Dis.* **160**:954-959.

Ehrlich, G.D., Davey, F.R., Kirshner, J.J., Sninsky, J.J., Kwok, S., Slamon, D.J., Kalish, R., and Poiesz, B.J. (1989) "A polyclonal CD4+ and CD8+ lymphocytosis in a patient double infected with HTLV-I and HIV-1: a clinical and molecular analysis." *Amer. Jour. Hema.* 30:128-139.

Ehrlich, G.D., Glaser, J.B., LaVigne, K., Quan, D., Mildvan, D., Sninsky, J.J., Kwok, S., Papsidero, L., and Poiesz, B.J. (1989) "Prevalence of human T-cell leukemia/lymphoma virus (HTLV) type II infection among high-risk individuals: type specific identification of HTLVs by Polymerase Chain Reaction." *Blood* 74:1658-1664.

Wolinsky, S.M., Rinaldo, C.R., Kwok, S., Sninsky, J.J., Gupta, P., Imagawa, D., Farzadegan, H., Jacobson, L.P., Grovit, K.S., Lee, M.H., Chmiel, J.S., Ginzburg, H., John J. Sninsky, Ph.D. Kaslow, R.A., and Phair, J.P. (1989) "Human immunodeficiency virus type 1 (HIV-1) infection a median of 18 months before a diagnostic Western Blot: evidence from a cohort of homosexual men." *Ann. Int. Med.* 111:961-972.

Ehrlich, G.D., Glaser, J.B., Abbott, M.A., Slamon, D.J., Keith, D., Sliwkowski, M., Brandis, J., Keitelman, E., Teramoto, Y., Papdisero, L., Simpkins, H., Sninsky, J.J., and Poiesz, B.J. (1989) "Detection of Anti-HTLV-I tax antibodies in HTLV-I enzyme-linked immunosorbent assay-negative individuals." *Blood* 74:1066-1072

PUBLICATIONS: (cont.)

Kwok, S., Mack, D.H., Sninsky, J.J., Erlich G.D., Poiesz, B.J., Dock, N.L., Alter H.J., Mildvan, D. and Grieco, M.H. "Diagnosis of Human Immunodificiency Virus in Seropositive Individuals: Enzymatic Amplification of HI:V Viral Sequences in Peripheral Blood Mononuclear Cells," in *HIV Detection by Genetic Engineering Methods* (Luciw, P.A. and Steimer, K.S., eds.). Marcel Dekker, Inc., New York, 1989, p. 243 - 255.

Jackson, J.B., MacDonald, K.L., Cadwell, J., Sullivan, C., Hanson, M., Sannerud, K.J., Stramer, S.L., Fildes, N.J., Kwok, S.Y., Sninsky, J.J., Bowman, R.J., Polesky, H.F., Balfour, Jr., H.H., and Osterholm, M.T. (1990) "Absence of human immunodeficiency virus infection in Western Blot indeterminate blood donors." *N. Eng. Jour. Med.* 322:217-222.

Kwok, S., Kellogg, D., Levenson, C., Spasic, D., Goda, L., and Sninsky, J.J. (1990) "Effects of primer-template mismatches on the polymerase chain reaction: Human immunodeficiency virus type 1 model studies." *Nucl. Acids Res.* 18:999-1005.

Sninsky, J.J. and Kwok, S. (1990) "Detection of human immunodeficiency viruses by the Polymerase Chain Reaction." *Arch. Pathol. Lab. Med.* 114:259-262.

Jackson, J.B., Kwok, S.Y., Sninsky, J.J., Hopsicker, J.S., Sannerud, K.J., Rhame, F.S., Henry, K., Simpson, M., and Balfour, H.H., Jr. (1990) "Human immunodeficiency virus type 1 detected in all seropositive symptomatic and asymptomatic individuals." *J. Clin. Microbio.* 28:16-19.

Papsidero, L., Swartzwelder, F., Sheu, M., Montagna, R., Whrlich, G., Bhagavati, S., Dosik, H., Sninsky, J. and Poiesz, B. (1990). "Immunodetection of hiuman T-cell lymphotropic virus type I core protein in biological samples by using a monoclonal antibody immunoassay." *Journal of Clin. Microbio.* 28:949 - 955.

Kwok, S., Lipka, J.S., McKinney, N., Kellogg, D.E., Poiesz, B., Foung, S.K.H., and Sninsky, J.J. (1990) "Low incidence of HTLV infections in random blood donors with indeterminate Western Blot patterns." *Transfusion* (in press).

Kwok, S., Gallo, D., Hanson, C.V., McKinney, N., Poiesz, B., and Sninsky, J.J. (1990) "High prevalence of HTLV-II among intravenous drug abusers: PCR confirmation and typing." *AIDS & Human Retroviruses* 6:559-563.

Kellogg, D.E., Sninsky, J. and Kwok, S. (1990) "Quantitation of HIV-1 Proviral DNA Relative to Cellular DNA by the Polymerase Chain Reaction." *Analytical Biochemistry* 189:202-208.

Erlich, H.E., Gelfand, D. and Sninsky, J. (1991) "Recent Advances in the Polymerase Chain Reaction". *Science* 255:1643-1651.

Busch, M.P., Eble, B.E., Khayam-Bashi, H., Heilbron, D., Murphy, E.L., Kwok, S, Sninsky, J.J., Perkins, H.A., Vyas, G.N. (1991) "Evaluation of Screened Blood Donations for Huma Immunodeficiency Virus Type 1 Infection by Culture and DNA Amplification of Pooled Cells". *The New Eng. Jour. of Med.* 325:1-5.

NOTE THAT SUBMITTED MANUSCRIPTS, CHAPTERS, ABSTRACTS,

AND PATENTS HAVE NOT BEEN INCLUDED.

AVAILABLE UPON REQUEST

12/11/90

EXHIBIT B

ATTORNEY'S EYES ONLY
RESTRICTED

RMS 71087

GENETICS

THE BOSTON GLOBE MONDAY SEPTEMBER 7, 988

DNA test exceeding hopes – in many fields

PCR-working in AIDS research, forensics, archeology

By Judy Foreman
Globe Staff

Two and a half years ago, four gay men infected with the AIDS virus – a fraction of the nearly 5,000 men being studied through an AIDS research project – appeared to do what no one thought possible: lose the antibodies to the virus they had acquired.

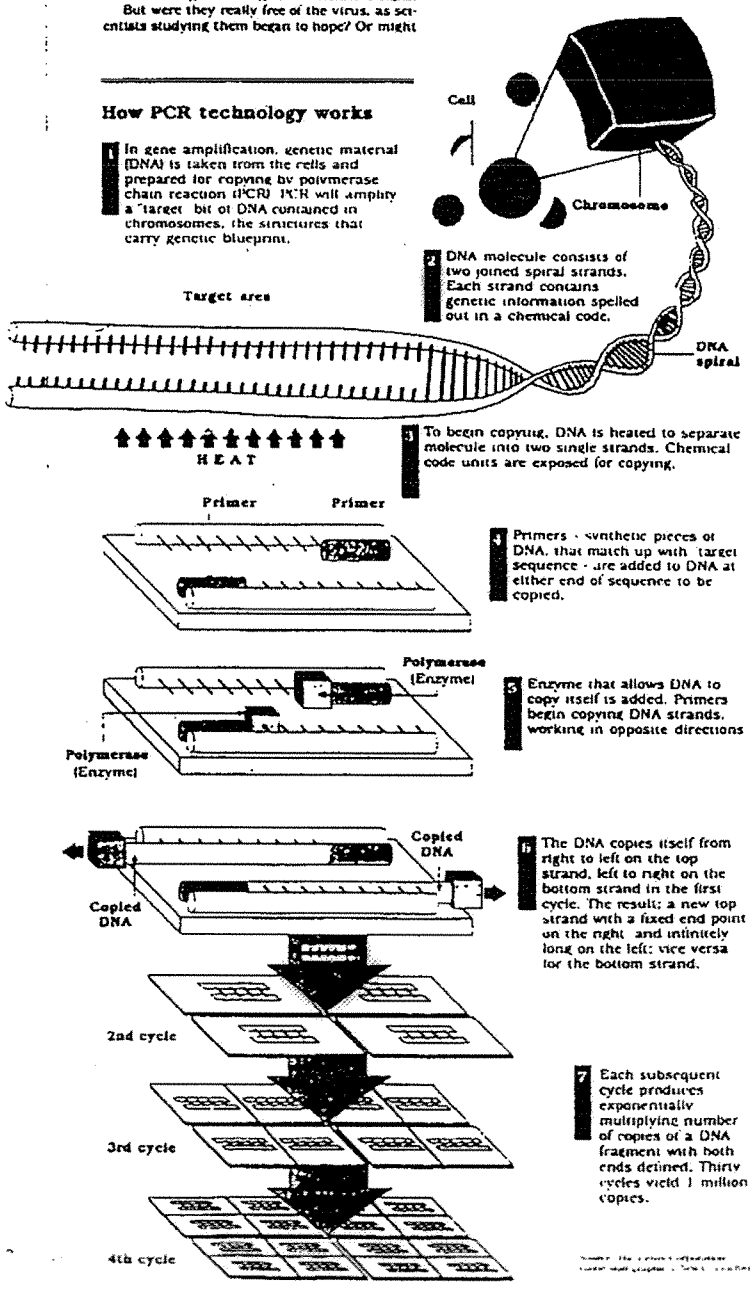
All four were healthy, and researchers were unable to "culture," or grow, virus from their blood – both good, though not conclusive signs.

But were they really free of the virus, as scientists studying them began to hope? Or might traces of virus still lurk deep inside their cells, undetectable by available tests?

When the cases surfaced, there was no good way to tell. Today, there is, through a gene amplification process called PCR, for polymerase chain reaction. It's not perfect, but PCR is already exceeding expectations, not just among

DNA, Page 31

How PCR technology works



DNA test exceeds hopes

■ DNA

Continued from Page 29

AIDS researchers, but among forensic scientists, genetic disease researchers, even archeologists.

Simply put, PCR allows researchers to take a tiny fragment of DNA, or genetic material, define a "target" area to be duplicated, then add an enzyme that causes millions of copies of the target area to be made, all within about three hours.

In the puzzling case of the four gay men, for instance, researchers Homayoon Farzadegan and Alfred J. Saah of Johns Hopkins University determined through PCR that all four did indeed still carry the AIDS virus – six to 18 months after the standard tests indicated they had lost the antibodies, a major sign of infection. (With PCR tests repeated later, however, one of the four showed no trace of virus and now appears to be antibody-free.)

And AIDS testing is just the beginning. Last week, PCR showed its potential in a different field, prenatal diagnosis.

Another team from Johns Hopkins, collaborating with the Cetus Corporation in Emeryville, Calif., which developed and patented the PCR technology, described the technique's usefulness in diagnosing two inherited blood diseases, sickle cell anemia and beta-thalessemia. The work appeared last week in the New England Journal of Medicine.

Conventional tests for sickle cell anemia require one to two weeks just to grow fetal cells to produce enough DNA to test. Then, it can take another week to cut the DNA with enzymes, run it through gels, blot it onto filter paper, label it with radioactive probes and read the results from X-ray film.

With PCR, says Dr. Henry A. Erlich, Cetus' director of human genetics, the process takes hours.

Used in criminal cases

And a month ago, PCR proved useful in yet another field, forensics. With Edward Blake of Forensic Sciences Associates in Richmond, Calif., Erlich amplified DNA in a 10-year-old semen specimen, showing beyond a doubt, he says, that Gary Dotson had been wrongly convicted in Illinois of raping Cathleen Crowell Webb in 1975. Webb in 1985 recanted her testimony. Subsequent efforts to analyze the semen with conventional DNA tests failed because the DNA over time had been broken down by heat, moisture or bacteria into fragments so tiny the tests would not work.

But PCR, says Erlich, showed that the semen sample did not match Dotson's but did match Webb's boyfriend, thus supporting Webb's recantation. On the basis of the PCR test, Dotson has requested a full pardon from Gov. James Thompson, who is expected to reviewthe case in October.

Because of PCR's ability to produce large amounts of usable DNA from degraded samples, the FBI last week said it was "actively pursuing the technique to make it applicable to forensic science."

Two commercial forensic firms, Cellmark Diagnostics and Lifecodes, Inc., have indicated they hope to add PCR to conventional DNA analysis in paternity and criminal cases.

Even in anthropology and archeology, PCR is yielding insights. At the University of California at Berkeley, researchers led by Allan C. Wilson amplified DNA from a woolly mammoth that lived 40,000 years ago in Siberia, showing it was related to modern elephants. They have identified as human a 7,000-year-old brain found in a Florida peat bog.

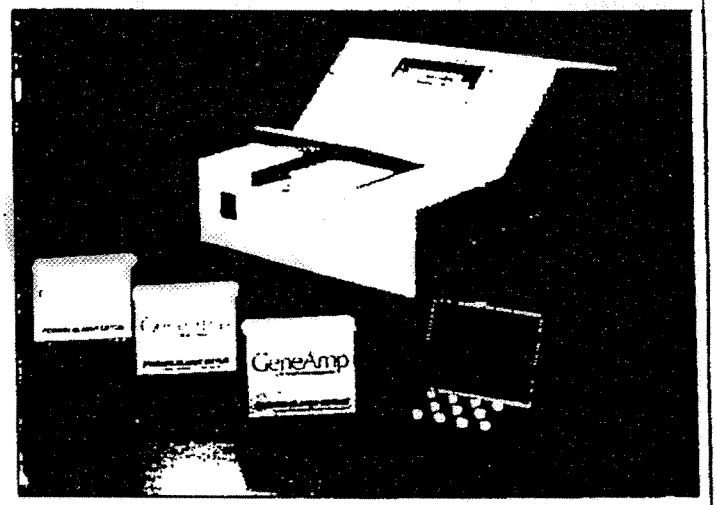
It's not perfect

Like any new technology, of course, PCR has its flaws.

Precisely because it so sensitively detects tiny fragments of DNA, the test might yield "false positives" if laboratory dishes should become contaminated with DNA from other experiments. And with AIDS testing, cautions Dr. Robert C. Gallo, head of the tumor cell biology laboratory at the National Cancer Institute, PCR could pick up stray, damaged portions of AIDS virus in somebody who did not carry the whole virus.

Researcher Henry A. Erlich with DNA dot blot.

Photos courtesy/Cetus Corporation



DNA Thermal Cycler performs duplicating process automatically.

There is also the possibility, others note, of false negatives. Because only a small sample of circulating blood is drawn for testing, AIDS-infected cells hiding in the liver, spleen or brain, for instance, might not be in the sample.

Despite such caveats, PCR's popularity is soaring. The technology has been patented only since July, and an $8,500 "DNA thermal Cycler" machine that performs the process automatically has been on the market only nine months, through a joint venture with Perkin-Elmer Corp., of Norwalk, Conn. But already the firm has 1,000 orders and a two-month waiting list. And 70 firms want licenses to use PCR technology for profit.

Until PCR came along, looking for a particular bit of DNA – perhaps a stretch containing a mutant gene, perhaps a stretch containing pieces of the AIDS virus – was like looking for a needle in a haystack, says Cetus scientist John Sninsky, noting that sometimes only one cell in a million is infected.

With PCR, researchers not only find that needle, but produce millions of copies of it in hours, making a haystack of needles.

With AIDS, the body makes antibodies only while the virus is active and "exposing" itself to the immune system. PCR, however, can detect hidden, inactive virus because it searches out the nucleic acid building blocks of viral DNA.

Similarly, in searching for disease-causing genes, PCR can amplify a segment of DNA that might contain these genes, providing ample material – quickly – on which to use more conventional tests for genetic defects.

The way it works

Essentially, PCR works like this. Researchers first take a fragment of DNA – from blood or hair or semen, etc. – and boil or "denature" it, a process that separates the doubled-stranded DNA into single strands. The strands, strings of chemical units known as nucleotide bases, are chemically complementary, meaning that a nucleotide on one automatically pairs with a particular nucleotide on the other strand. The two strands then lie head-to-toe, like nestling worms.

Next, researchers add two primers – synthetic bits of DNA that search out and chemically bond to a particular pattern of nucleotide bases along each strand of DNA. One primer binds to one DNA strand, one to the other strand. Each primer carries a "start" signal that tells the DNA to begin copying itself, taking advantage of DNA's natural ability to replicate.

This copying is greatly boosted by an enzyme called a DNA polymerase, which hooks onto the end of the primer. The enzyme used in the Cetus technology – the key to the whole process – is made by bacteria called thermus aquaticus (TAQ) that live in places like Yellowstone's hot springs. Unlike other enzymes, TAQ can withstand the heating and cooling required for PCR.

The first PCR reaction yields two identical doubled-strand stretches of DNA, the second yields four, the next, eight, and so on. As long as the initial quantities of primer and polymerase are sufficient, the process is self-repeating, each cycle yielding twice as much DNA as the cycle before. Thirty cycles yield more than a million copies.

Because each cycle in the chain reaction uses as a template the DNA with fixed end points (marked by the primers) created in the preceeding cycle, from the second cycle onward only the target area between the primers actually gets duplicated exponentially.

The resulting batch of DNA consists of discrete pieces of DNA, all identical copies of the target area. With this abundance of DNA, conventional tests can be used to answer researchers' questions: Does the target area contain pieces of the AIDS virus, does it contain disease-causing genes, do the genes match match those of a criminal suspect?

Conceived four years ago by Kary Mullis at Cetus, the PCR technology is already in use at Johns Hopkins for prenatal diagnosis of sickle cell anemia and beta thalessemia, and is coming into routine use at other major medical centers.

The technology, says Erlich of Cetus, will also give more precision to tissue-typing, the matching of donor and recipient tissues for to organ transplants. "It's also simpler, so it will be cheaper," he adds.

Dr. David L. Valle of Hopkins calls PCR "the biggest technological development in recent years" for geneticists and molecular biologists. In the last year, adds Hopkins geneticist Victor McKusick, it has been to identify a number of mutations in the gene that causes hemophilia, a bleeding disorder.

And combined with conventional technology, says McKusick's colleague, Alan Scott, PCR could make greatly aid efforts to map the human genome, an ambitious project to identify and locate precisely all the genes on human chromosomes. The technology, he says, will make the work go "10 times faster, and more importantly, allow it be automated. With PCR, you can do a day what would normally take a week."

In fact, he says, the uses for the technology are so broad that scientists "joke that PCR stands for 'People's Choice Reaction. Everybody's trying to use this for all things."

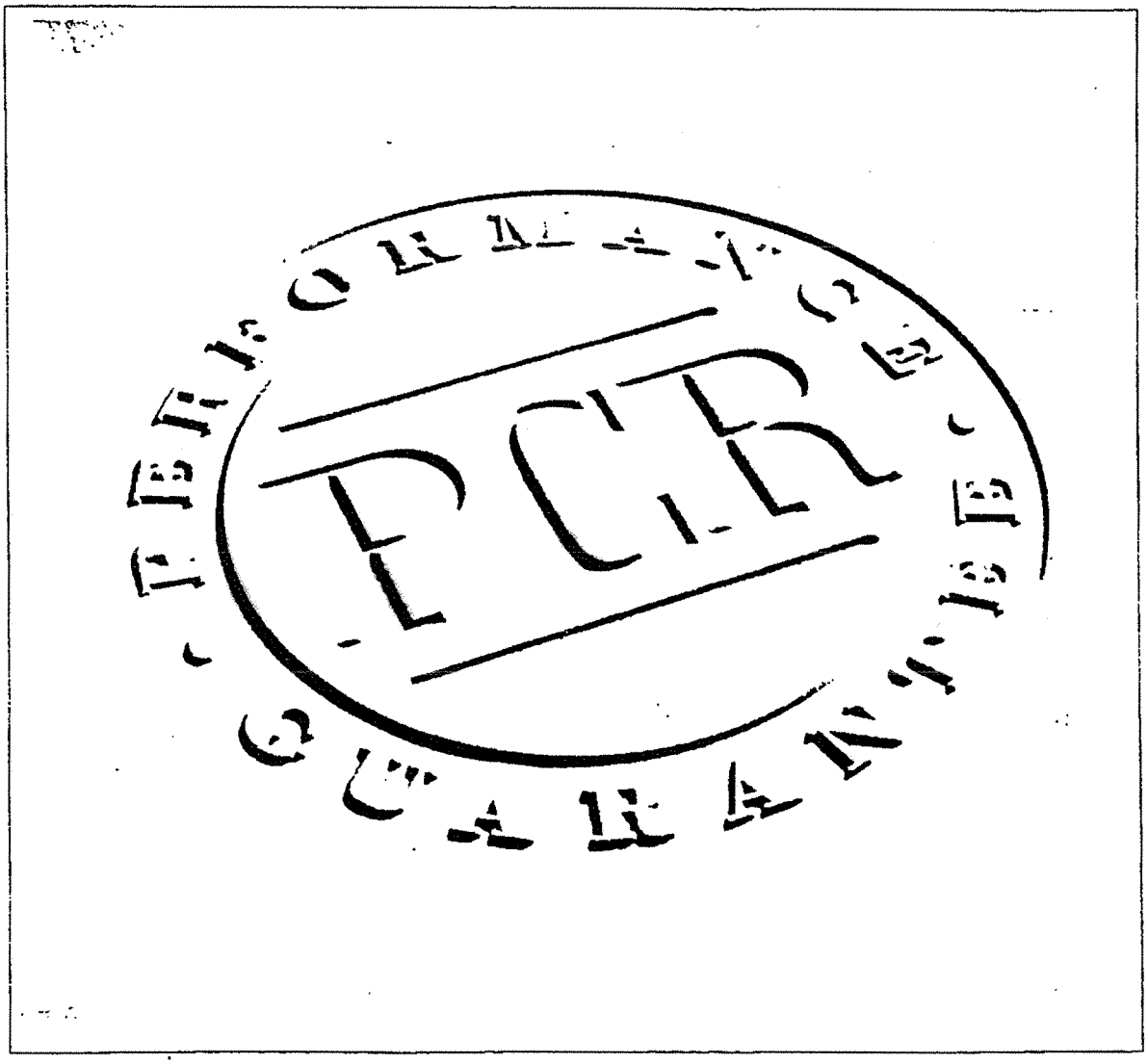
EXHIBIT C

ATTORNEY'S EYES ONLY
RESTRICTED

1991

PERKIN ELMER CETUS

Biotechnology Catalog



PE XPRESS

Your Source for Perkin-Elmer Cetus Instruments, Reagents, and Supplies

CALL TOLL FREE
1-800-762-4002

ATTORNEY'S EYES ONLY
RESTRICTED

RMS 71093

PERKIN ELMER CETUS

amplifications
A Forum for PCR Users

March 1990 Issue 4

Orienting Inserts in Recombinant DNA Molecules by PCR

Ronald F. Thomas, Ph.D. and John E. Newbold, Ph.D.
Department of Microbiology and Immunology
University of North Carolina
Chapel Hill, NC 27599

The generation of recombinant DNA molecules is widespread in current molecular studies. Clones and libraries of clones are commonly produced, and test fragments of DNA are often inserted into a variety of vectors for subsequent expression or assays relating to gene regulation. When either blunt-end ligation or ligation into a single restriction site is carried out, there is typically a need to determine the orientation of the DNA insert. Furthermore, one needs to verify that the molecules are recombinant and do not represent recircularized parent vector molecules. Traditionally, these determinations had to be made by sequencing or by restriction map analysis. PCR, however, offers a rapid and simple method for obtaining this information with both single-stranded and double-stranded recombinant molecules. In the case of the commonly used single-stranded vector, M13, PCR can be carried out directly on the phage following lysis of the virion. Double-stranded DNA molecules such as the M13 replicative form and the many prokaryotic and eukaryotic cloning vectors can be subjected to PCR after a small-scale DNA preparation (i.e., mini-preps).

The amplification protocol is essentially the same for both single- and double-stranded molecules, with primer selection being the key consideration. Consider the question of determining the orientation of small inserts with possible enhancer activity in an appropriate CAT plasmid. A pair of reactions is set up (Figure 1, A and B) with each reaction containing a first primer that will anneal to one strand of the parent vector at a convenient distance from the cloning site. In reaction A, the second primer used is one that will anneal to the coding strand of the insert, again at a convenient distance from the cloning site. Reaction B receives a second primer that will anneal to the complementary strand of the insert. When the

continued on Page 3

In This Issue: