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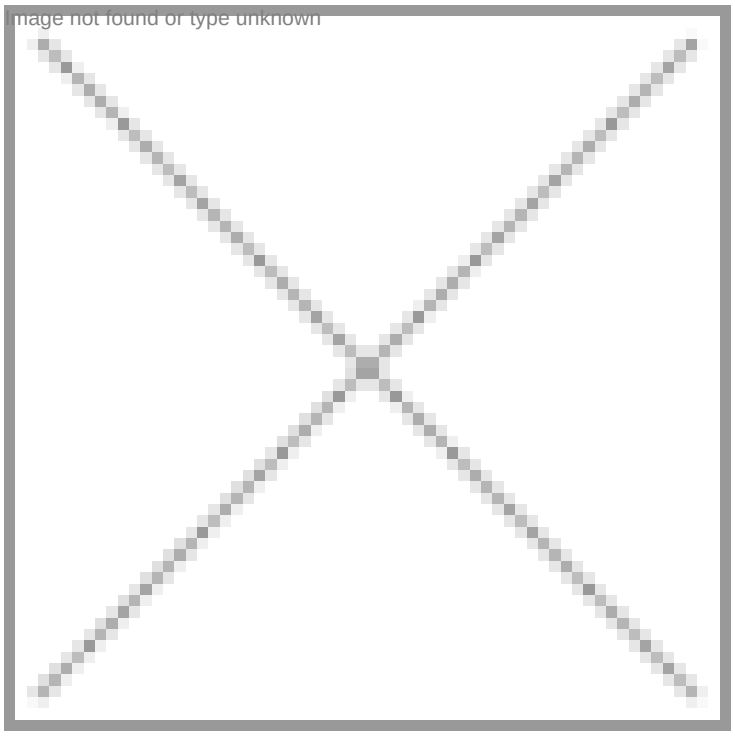
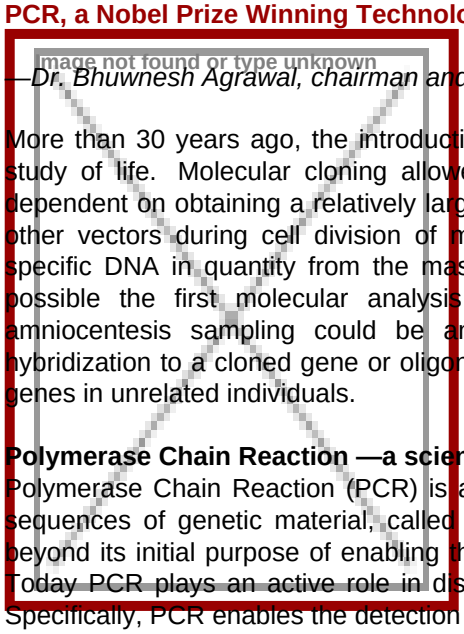
PCR, a Nobel Prize Winning Technology

10 March 2009 | News



PCR, a Nobel Prize Winning Technology



—Dr. Bhuwnesh Agrawal, chairman and managing director, Roche Diagnostics India

More than 30 years ago, the introduction of recombinant DNA technology as a tool for the life sciences revolutionized the study of life. Molecular cloning allowed the study of individual genes of living organisms; however this technique was dependent on obtaining a relatively large quantity of pure DNA. This depended on the replication of the DNA of plasmids or other vectors during cell division of microorganisms. Researchers found it extremely laborious and difficult to obtain a specific DNA in quantity from the mass of genes present in a biological sample. Recombinant DNA technology made possible the first molecular analysis and prenatal diagnosis of several human diseases. Fetal DNA obtained by amniocentesis sampling could be analyzed by restriction enzyme digestion, electrophoresis, southern transfer and hybridization to a cloned gene or oligonucleotide probes. However, southern blotting permitted only rudimentary mapping of genes in unrelated individuals.

Polymerase Chain Reaction —a scientific success story

Polymerase Chain Reaction (PCR) is a Nobel-prize winning technology used to amplify, or copy on a large scale, specific sequences of genetic material, called Deoxyribonucleic Acid, or DNA. The importance of this technology has moved far beyond its initial purpose of enabling the detailed analysis of DNA for molecular biology applications and gene sequencing. Today PCR plays an active role in disciplines as varied as archeology, forensics, genetics, histopathology, and medicine. Specifically, PCR enables the detection and analysis of DNA for purposes such as:

- Diagnosis of genetic conditions or illnesses

- Predicting how well a patient will respond to medical treatment
- Identifying viruses or pathogens
- Connecting a suspect to a crime
- Establishing maternity, paternity or other blood relation
- Determining identity

History of PCR technology

First described in the journal Science in 1985, PCR has become one of the most widely used techniques in molecularbiology and for good reason: from the daily practicalities of medical diagnosis to the courts of law, PCR takes the analysis of tiny amounts of genetic material to a new level of speed, precision and reliability. Because it is far simpler, faster, and less expensive than previous technologies for analyzing DNA (Southern Blot for example), PCR has "democratized" genetic research, putting a powerful research tool in the hands of all biologists, even those with a limited training in molecular biology. In 1993, the Nobel Prize for the conception of PCR was given to Kary Mullis. The technology was further developed and applied by the labs of Henry Erlich and David Gelfand, both now working at Roche, and other teams of scientists at Cetus. The full history of the technology's development and application is marked by an extraordinary collaboration of scientists working in a corporate setting, working together to identify and overcome the obstacles to the practical use of PCR for DNA analysis in research and medicine. In 1991, Roche acquired the rights to PCR technology from Cetus and then dedicated significant resources to accelerate the technology's further advance. From its beginning as a simple but highly manual process to create many copies of a specific region of DNA using a test tube, enzymes a reagents, and a heating source to vary temperature, PCR is now a highly reproducible process that is almost completely automated and therefore simple enough to be applied to many fields of science.

Fields of application

During the last twenty years, PCR has radically changed the scientific world by making it possible to produce sufficientcopies of, or amplify, a portion of DNA for reliable analysis. The list of applications for PCR is lengthy and ever growing. For example, pathogenic organisms can be detected in miniscule amounts of blood, allowing reliable diagnosis and quantitation, thus enabling more rapid medical response.

Health and medicine

Health, medical treatment and the understanding of disease have all benefited tremendously from PCR technology. In anage where the genetic component of disease is increasingly apparent, PCR will continue to play an important role in medicine. In the late 1990s, the Human Genome Project, which produced the first complete sequence of the human genetic code, drew attention to the potential of understanding the DNA sequence for aiding the development of new drugs and diagnostics, and have uncovered important variations in the nucleotide bases or polymorphisms that make up our genes. These single nucleotide polymorphisms (SNPs) are associated with an increased risk of contracting a variety of common and complex disease, including various cancers and cardiovascular disease, and have become targets for developing new treatments to combat these diseases.

The ability of PCR and gene cloning (a procedure that uses organisms such as bacteria and yeast to reproduce genes) to produce sufficient quantities of the DNA code made them instrumental in large-scale sequencing efforts. In the ongoing research of the genome to uncover more information about which genes play a role in disease and why some patients are more susceptible to disease than others, PCR remains critical.

For patients today, PCR's ability to detect genetic variations and mutations has become important for the treatment of such life-threatening diseases as cancer. The term "personalized medicine" refers to the goal of ensuring that each individual patient gets the treatment that will be most successful for him or her. The most innovative new therapies for cancer, drugs such as Gleevec (Imatinib) for chronic myeloid leukemia, Tarceva (erlotinib) for lung cancer, and Herceptin (trastuzumab) for breast cancer, are examples of targeted therapy that are dependant on knowing which gene mutation is present or which genes are expressed in the patient's cancer tumor. PCR enables the application of personalized medicine by providing reliable and efficient analysis of tumor DNA.

In general PCR facilitates the practice of, "translational medicine", which combines knowledge gathered on the molecular level with that of a patient's care, based on the direct link between laboratory analysis and practical clinical assessment and management of individual patients.

PCR is an essential tool for improving human health in the diagnosis of infectious disease. Physicians and researchers can analyze minute amounts of biological material to track down the source of a viral infection. The use of PCR to detect harmful pathogens is not limited to day-to-day medical diagnosis; because immediate response to large-scale epidemics or containment of contanimated water supplies in extreme situations (catastrophic medicine) is critical, the ability to confirm the presence of a pathogen or virus rapidly and precisely is very important.

Forensic science

Since the first landmark US case of PCR-based genetic analysis in 1986 by the lab of Henry Erlich, PCR has vastlyimproved police ability to confirm or exonerate suspects based on their genetic code, which is as unique as a fingerprint. In contrast to

the earlier techniques for comparing DNA using tissue samples found at a crime scene with samples from suspects, PCR can amplify DNA using miniscule amounts of biologic material, including, but limited to, samples of blood, semen, hair, or saliva.
Enabled by PCR, forensic science today can establish a genetic fingerprint from biological traces from dental molds,cigarette butts, eating utensils, and licked envelopes. PCR has the additional benefit of providing results rapidly, usually within 24 hours, which enables police investigation to move forward quickly.
PCR genetic analysis comparing forensic specimens with genetic profiles stored in a database of those previously convicted has solved a number of crimes without a known suspect. PCR genetic analysis has also been able to demonstrate the innocence of the wrongfully convicted in hundreds of cases. PCR can also be used to locate traces of marijuana or other illegal substances. The role of PCR in investigation was underlined by the U.S Justice Department's prediction that by 2010 investigators will be using portable PCR instrumentation to characterize DNA at a crime scene.

Archeology and developmental biology
PCR has also become useful in some scientific disciplines not directly linked with molecular biology. For example, archeologists have found PCR effective for determining relationships between ancient civilizations and modern man.
In addition, PCR can provide a new level of information about individuals from early history through genetic analysis of ancient tissue specimens, for example from mummies. Researchers can use PCR to correlate information from history, for example what the historic record says about a Pharaoh, to the facts apparent from genetic information.
Evolutionary biologists use PCR routinely to chart the evolution of plant or animal species. In the ongoing study of human evolution and anthropology, PCR has helped clarify the genetic differences between Neanderthal man and modern man, which although far greater than the miniscule differences between modern individuals from different parts of the globe, are only half that of the genetic differences that separate us from our primate ancestor, the chimpanzee. Important inferences about human evolution can also be obtained by analyzing genetic differences among modern human populations.

Food production and safety
PCR is effective for analyzing the genetic information of the food we eat, in that it is able to differentiate between genetically modified plants and traditional plant species. In addition, PCR is highly effective in locating pathogens like Salmonella and Listeria in food as well as confirming water contamination, aiding the effort to improve food and water safety worldwide.

PCR technology development
Although simple, significant technological challenges had to be overcome before PCR could be used reliably and reproducibly. The newest forms of PCR respond to scientific and technical needs, for example, reverse transcriptase or RT PCR allows scientists to amplify RNA (Ribonucleic Acid) in the same way they copy DNA. Specialized enzymes enable the amplification of DNA strands that are several thousand bases in length; others produce amplification at a very high level of accuracy. A form of the technology that monitors PCR amplification in real time extends scientists' ability to make truly quantitative measurements.
Other technical advances include "hot start" systems and automatic cycling systems make DNA copying extremely specific and precise. As in other areas of the life sciences, PCR can also be miniaturized, making the technology even more accessible. The development of PCR technology today can be described as a continual process of overcoming the technical challenges with efficient solutions.
Going ahead, PCR will be used to find genetic constellation that are susceptible or resistant to certain diseases. It will also help define which drugs and dosages will be optimal for us.
For example, K-RAS is a gene that codes for a protein that plays an important role in the epidermal growth factor receptor (EGFR) pathway – a complex signaling cascade that is involved in the development and progression of cancer.
The K-RAS gene is a defining factor in looking at whether a patient with colorectal cancer can benefit from EGFR inhibitors,a therapy which prevents growth signals from entering the cells, thus stunting the growth of the tumor. Patients with a mutated K-RAS gene (35-45% of metastatic colorectal cancer patients) do not benefit from certain EGFR inhibitors like Erbitux.
Recent studies in non-small cell lung cancer have shown that some patients carry somatic mutations in the EGFR gene. These mutations may correlate with responsiveness to the EGFR tyrosine kinase inhibitors, with some mutations having a sensitizing effect and others being linked to resistance.
More and more complex disease mechanism will be understood which are often polygenetic in nature. They will help us to screen high risk, prognosticate disease and optimize treatment, for eg, in various forms of cancer and metabolic disease. This can be done using PCR or microarrays.
In few years from now, a genome would probably cost as low as $1,000, thereby facilitating individualized treatment and personalized healthcare with PCR technologies. This also will bring about better understanding of epigenetic phenomena, which represent modifications of the genetic sequences by methylation.
Genomics will not only continue to develop into a routine technology but also a cutting edge tool to new areas of biology.