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â€œPCR has become the cornerstone of modern molecular biologyâ€?

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—Dr Carl Wittwer, Professor of Pathology, University of Utah Medical School, Salt Lake City, Utah, USA

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Polymerase Chain Reaction (PCR) is a method that allows exponential amplification of short DNA sequences (usually 100 to 600 bases) within a longer double stranded DNA molecule. First discovered in 1983, PCR has now become a common and indispensable method that researchers and scientists use in research labs for a variety of different applications. From its initial application in DNA amplification and genotyping, PCR is now applied to fields such as forensics, archaeology and even in food and beverage industries.

A decade old, the Roche LightCycler 480 system is one that has been widely used in research labs worldwide. Since its invention, the LightCycler system has evolved into a sophisticated system that comprises five excitation channels and six detection channels, where researchers can observe and track the progress of experiments in real time to ensure that they get an improved PCR result.

In an email interview with BioSpectrum, Dr Carl T Wittwer, the inventor of the LightCycler system, shares his views about the evolution of the system and where he sees PCR progressing in the next decade. Professor of Pathology at the University of Utah Medical School, Dr Wittwer developed rapid-cycle PCR techniques for DNA amplification in the early 1990s and adapted flow cytometry optics to thermal cycling for the read-time monitoring of PCR in the mid 1990s.

What are the major breakthroughs achieved with PCR over the last decade?

PCR has enabled many breakthroughs in research and clinical medicine. The ability to amplify and duplicate genetic material within a very short period has made it possible for researchers to unravel the human genome and understand infectious and hereditary diseases. For example, with PCR we have been able to understand genetic contributions to Parkinson’s disease better over the last decade. Previously unsuspected genes have also been discovered that contribute to acute myeloid

leukemia, a particularly deadly form of leukemia through PCR.
Over the years, many variants of PCR have been developed. One of them is quantitative real-time PCR, more commonly know as qPCR. With qPCR, the exponential phase of PCR is monitored, allowing determination of the initial amount of target. The contributions of real-time PCR systems are now indispensable in the research lab. PCR technology today is being applied across different industries and fields of study, including forensic science, virology, food microbiology and even environmental monitoring.
Roche provides research laboratories all over the world with a series of different real-time PCR systems to suit their needs. With the LightCycler 2.0 and LightCycler 480 real-time PCR systems, researchers have access to both rapid carousel-based and high-throughput systems. The LightCycler introduced many “firsts” to real-time PCR, including rapid cycling, SYBR Green I, dual hybridization probes, single hybridization probes, and melting analysis. As a rapid, open system, it remains the most user-friendly real-time instrument for assay and technique development. The first genetic tests to receive FDA approval in the US (F5 and F2) were on the carousel LightCycler in 2002, setting the standard for other companies to follow.
The extreme sensitivity of real-time PCR, with the ability to detect even a single nucleic acid molecule, makes it possible to detect fetal DNA in maternal serum for noninvasive prenatal diagnosis. Digital PCR is a recent modification where the sample volume or concentration is reduced so much so that each reaction only contains one starting template. Emulsion PCR creates millions of nanoliter PCRs within oil, a process that is central to most next-generation sequencing methods.

Who are the major players in real time PCR systems? What is the current market size of real time PCRs (number of PCR systems sold per year?)
Roche introduced the LightCycler in 1998, the first system to automate absolute quantification and display PCR in real-time as the reaction progresses. To date, over 7,000 LightCycler systems have been sold. The market size for real-time PCR continues to expand in research and medical diagnostics. Based on rapid cycle PCR, carousel LightCyclers can complete PCR in 15-30 minutes, providing rapid turn around for critical clinical settings and sequential research. In situations where high throughput is more important than turn around time, the LC480 allows amplification on 96 or 384-well plates. A 1536-well LightCycler (again a first in real-time PCR) will be introduced in 2009.

What are the factors that drive PCR evolution?
The need for speed, accuracy and cost containment are factors that drive PCR evolution. Initially, finding a polymerase that withstood the high temperatures involved in the duplication of DNA was critical. The discovery and usage of the heat-resistant Taq polymerase in 1988 greatly revolutionized the PCR technique by allowing automated temperature cycling without reagent addition. The process of PCR suddenly became much simpler and more accessible. However, quantitative data was still difficult to obtain, and the process remained qualitative until the introduction of real-time PCR instruments in the mid 1990s. By monitoring fluorescence each cycle of PCR, the precise amount of starting template could be determined, allowing true quantification. Quantification was critical for mRNA quantification in research and viral load determination in clinical applications. Time consuming and expensive microbial diagnostics began to be replaced by faster, more accurate real-time PCR assays.
The next advance was melting analysis, a process that goes beyond conventional real time PCR. In addition to monitoring fluorescence once each cycle, fluorescence is monitored continuously as the temperature changes so that hybridization can be followed. Both PCR product hybridization with SYBR Green I and probe hybridization for genotyping can be monitored at the end of PCR. As melting technology became better and better, high resolution melting analysis was introduced as the latest method for product analysis. Labeled probes were no longer necessary for genotyping and entire PCR products could be scanned for single base changes in only one copy of diploid DNA.
The LightCycler real-time PCR systems are a good example of how the PCR technique has evolved. The LightCycler 1.5real time PCR system was the first to monitor the hybridization process through melting curve analysis and automate absolute quantification. The LightCycler 2.0 system advanced multicolor analysis to provide researches with up to six colours of analysis. As real-time PCR evolved and high throughput analysis became more important, Roche developed the LightCycler 480 system to accommodate both 96 and 384-well microtiter plates. The variable plate formats available on the LightCycler 480 meant that scientists could exchange thermal block cyclers in a few minutes without having to recalibrate the instrument.

What will be the future for PCR in the next decade?
PCR will become faster, more precise, and more affordable with lower sample volumes. For most applications, 5-10 minPCR is feasible. Unlabeled probes will replace labeled probes. High resolution melting will expand into additional applications such as sequence matching for transplantation testing. The flexibility and wide application base of PCR are difficult to beat. For specific applications, alternative technologies will excel. For example, arrays for copy number variations are very powerful, and provide data that is difficult to obtain by PCR alone. However, PCR has become the cornerstone of modern molecular biology. It will be with us forever.

Narayan Kulkarni in Singapore