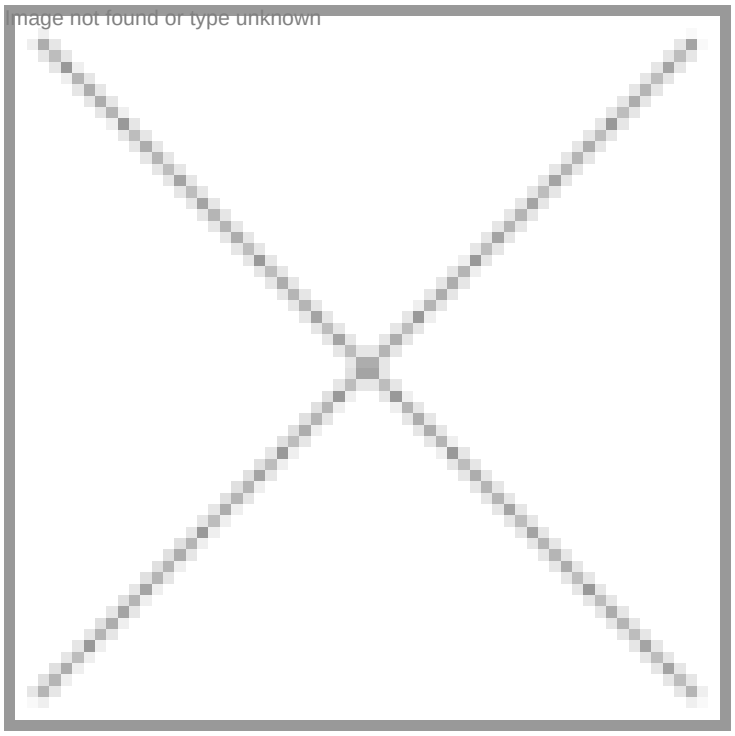
BioSpectrum the business of Bio & Health Sciences

PCR Changes the World of Molecular Biology

10 March 2009 | News



PCR Changes the World of Molecular Biology

Since its discovery, PCR has established itself as the most reliable technique that is accelerating biotech research and gaining prominence in almost all areas of life sciences.

The discovery of polymerase chain reaction or PCR has forever changed the molecular biology world. It is an indispensable research technique capable of producing billions of copies of a DNA fragment from just few copies, in less than two hours and is used for a variety of medical and biological applications from basic gene sequencing, diagnosis of hereditary diseases, the identification of genetic fingerprints, the detection and diagnosis of infectious diseases to the creation of transgenic organisms.

Following its invention 26 years ago, PCR has been adapted extensively for numerous molecular biology applications. Gene expression analysis by reverse-transcription quantitative PCR (RT-qPCR) has been a key enabling technology of the post-genome era. PCR is also the lone technique that helped the synthetic oligonucleotide business become a thriving industry today.

PCR suppliers in India

Players with direct presence

- Bio-Rad
- Eppendorf
- Roche

Players with indirect presence

- ABI (Through Labindia)
- Bioneer (Krypton Biomedicals)
- Cepheid (Through Biotron Healthcare)
- Stratagene (Through Genetix)
- Takara (DSS Imagetech)
- Techne (Scientech, Carebio medicals)

The invention

Kary Mullis, who earned a PhD in biochemistry from University of California, Berkeley in 1973, conceived PCR as a meansto amplify a specific locus of interest on the human genome in 1983 .

After conceptualizing PCR, Kary labored for a number of months to work out experimental conditions. Since thermostable polymerases were not yet available, it was necessary to add Klenow after each thermal cycle, adding to the tedium of development. There were many failures and many reasons why PCR should not work. Ignoring the doubts of many, the scientist was able to perform his first successful experiment on December 16, 1983. A patent for PCR was awarded to Cetus Corporation, where Mullis worked when he invented the technique in 1983. The Taq polymerase enzyme was also covered by patents. Perkin-Elmer partnered with Cetus to commercially introduce a thermal cycler in the market. This platform was based on compressor driven refrigeration technology. Few years later, pharmaceutical major Hoffman La Roche purchased the rights to the patents in 1992 and currently holds those that are still protected.

Evolution of technology

As PCR introduced capabilities to identify, manipulate, and amplify DNA, research possibilities flourished. The detection of genetic mutations, the ability to detect the presence of previously unknown genetic material, as well as the ability to analyze degraded DNA, all became common practice. For example, diseases such as muscular dystrophy and HIV could be detected and diagnosed with the use of PCR.

As scientists grew more familiar with the technique of PCR, they began to expand on the utility of the method. And therehave been a lot of improvements in the technology, experimental design, and data analysis.

During the late 1980s PCR was used to measure the quantity of DNA present in a reaction, generating the term “quantitative PCR” or more simply, q-PCR. This technique further improved PCR by the isolation of Taq Polymerase in the early 1990s. qPCR and, more specifically, real-time qPCR has become a routine and robust approach for measuring the expression of genes of interest, validating microarray experiments and monitoring biomarkers. The use of real-time qPCR has nearly supplanted other approaches like Northern blotting and RNase protection assays.

PCR technology and method has today reached a mature stage of development and implementation. This technique further improved PCR by the isolation of Taq Polymerase in the early 1990s. The heat stable polymerase could remain active through many cycles of heat required for amplification and created the demand for faster cycling. Russell Higuchi and associates developed a system to monitor the amplification of DNA simultaneously to the reaction. The system involved ethidium bromide, a thermal cycler to irradiate samples with UV light, and a camera to record fluorescence.

“The introduction of Peltier-based temperature controlled blocks followed by the introduction of interchangeable blocks, multiple blocks, low reaction volume blocks, blocks with high ramping rates, and finally temperature gradient blocks led to global adoption of PCR. But, the most radical innovation in this space was the development of ‘real-time’ platforms that measure the amount of PCR product made at the end of each cycle. Real-time PCR platforms and the FRET- and SYBR Green-based chemistries enabling the same have become cornerstones of modern genomics, especially for gene expression and genetic analysis applications,” said Subramanian Dharmaraj, senior marketing manager, genomics division, Labindia.

In the early 1990s, fluorogenic dual labeled probes were developed as a means to practice q-PCR. In conjunction with fluorescent probes, PCR had further evolved into a sensitive quantification tool useful for the detection of any desired genetic element. As a result, the ability to measure gene expression and practice genotyping quickly became trivial and widespread throughout the biotechnology industry. Now, with the recent development of new dyes and quenchers such as the series of

Black Hole Quencher, CAL Fluor and Quasar dyes, the possibilities for PCR are seemingly endless.

Market

The real-time PCR market in itself has come a long way, since the introduction of the first commercial real-time PCR platform by Applied Biosystems. The focus has gradually changed from hardware to complete end-to-end workflow-based solution. Gradually, other players also started penetrating the market by introducing more user-friendly and flexible products.

There are over 15 companies operating in this space globally. Applied Biosystems has the highest share of the market with Bio-Rad, Stratagene, Eppendorf and Roche being the other major stake holders in the basic research market. Additionally, Corbett, Cepheid and Techne are also trying to make inroads into this market. As further innovation in instrument technology has been slowing down over the last few years, one can expect that further growth in this market will be mainly driven by innovative reagents and consumables for the next few years.

The use of molecular techniques in all segments of the clinical, diagnostic and testing markets has been growing by leaps and bounds over the last few years. This is the primary driver for the growth in the PCR market at present. Major players in PCR market in India include Applied Biosystems, Eppendorf, Bio-Rad, Stratagene, Techne, Corbett and Cepheid.

Technology upgradations

During the developmental stage of this product line, real-time PCR was perceived merely as an optical upgrade of conventional PCR. But as more sophisticated applications were enabled by the real-time PCR technique, instrument manufacturers also realized that the technique offered great potential and the need for rapid improvements in their platforms. As a result, hardware features including the light source, the detection systems etc. were rapidly upgraded to keep pace with the application development without diluting the cost to performance ratio. Innovative approaches such as virtual filters were also implemented to deconvolute complex fluorescent dye spectra thus achieving better signal to noise ratios.

Even though, there are various real-time PCR chemistries available, two chemistries are widely used by the scientists all over. These are FRET-based TaqMan technology and SYBR Green chemistry which is dependent on SYBR Green's ability to bind any double-stranded nucleic acid molecule. TaqMan chemistry is extremely accurate and highly specific and considered to be the gold standard for real-time PCR experiments. However, with proper optimization SYBR green based methods can also work well.

Having sensed the need for speed and reliability, technology providers have started offering ready-made off-the-shelfassays, customized assays and optimized reagents enabling sample preparation from a wide range of specimens. As of today, the entire focus has shifted to provide complete work flow solutions to researchers. This has allowed scientists to focus on the science and discovery rather than the technique.

The most recent and novel innovation in this area has been the adoption of the microarray format for running real-time PCR experiments. This format allows researchers to interrogate the expression level of hundreds of genes simultaneously. With increasing number of scientists wanting a much closer view of gene expression at the pathway and individual biological process level, which typically involves a few hundred genes, these real-time PCR based low density arrays have already become a very popular tool. These arrays are also becoming an incredibly powerful tool to analyze global gene expression changes in the entire miRNA gene set. The future of PCR remains bright as the technology becomes more rapid, cost-effective, easier to use, and capable of higher throughput.

Jahanara Parveen