ADVANCES IN PCR TECHNOLOGY

Lloyd H. Lauerman, Washington State University[1]

ABSTRACT

Since the discovery of the polymerase chain reaction (PCR) 20 years ago an avalanche of scientific publications have reported major developments and changes in specialized equipment, reagents, sample preparation, computer programs, and techniques generated through business, government and university research. The requirement for genetic sequences for primer selection and validation has been greatly facilitated by development of new sequencing techniques, machines, and computer programs. Genetic libraries, such as, GenBank, EMBL, or DDBJ continue to accumulate a wealth of genetic sequence information for development and validation of molecular-based diagnostic procedures concerning human and veterinary disease agents. Mechanization of various aspects of the PCR assay, such as, robotics, microfluidics, and nanotechnology has made it possible for rapid advancement of new procedures. Real-Time PCR, DNA microarray, and DNA chips utilize these newer techniques in conjunction with computer and computer programs. Instruments for hand-held PCR assays are being developed. The PCR and RT-PCR assays have greatly accelerated the speed and accuracy of diagnoses of human and animal disease, especially of the infectious agents that are difficult to isolate or demonstrate. The PCR has made it possible to genetically characterize a microbial isolate inexpensively and rapidly for identification, typing, and epidemiological comparison.

TEXT

The polymerase chain reaction (PCR) was discovered by Mullis in 1983 (Mullis et al, 1986, 1987) and he received a Nobel Prize for this achievement a decade later. Awareness of the significance of the PCR technique had spread throughout the scientific community of the world by the end of the 1980's and scientific articles were being published concerning the various uses of the PCR technique. By 1990 there was a considerable pool of 16S RNA gene sequences, which had been generated for phylogeny of microorganisms and these sequences could be used for primer selection by sequence alignment. New sequencing techniques, sequencing machines and computer programs for manipulation of sequence information have been developed that have greatly accelerated the accumulation of genetic sequences for development of newer and better PCR assays. The use of the Internet has made it possible to access scientific information for comparison of genetic sequences from around the world. Genetic libraries (GenBank, EMBL, DDBJ) continue to accumulate a broad selection of genetic sequence information for development and validation of molecular-based diagnostic procedures concerning human and veterinary disease agents.

[1] Correspondence to: lhlauerman@mindspring.com

The initial thermal cycler used in our laboratory in 1990 was a Perkin-Elmer model 480, which had a refrigeration unit to speed the rate of cooling and did not have the heated lid technology. Nanotechnology was utilized from the beginning of these techniques. Simple computer hardware and software were built into the early thermal cyclers. The next generation of thermal cyclers had heated lids, no refrigeration (worked off ambient temperature using fans), and more memory for programs. With the development of Real-Time PCR the instruments became more elaborate and expensive. The use of standard computers and specialized computer software were necessary to run the Real-Time PCR assays. The Real-Time PCR technology requires new primers and fluorescent tagged probes to be developed that are much more expensive to prepare. The major advantage of Real-Time PCR over standard PCR assay is the one tube and one time handling with the reading of the reaction during the assay.

Initially the simple procedure of boiling was used to release DNA from the sample and to obtain a greater yield the phenol-chloroform technique was used. Less toxic chemicals were researched for release and harvest of the nucleic acids. Then DNA and RNA affinity materials were used for extraction of the nucleic acids. Initially the microcentrifuge and chemical hoods were the instruments necessary for DNA/RNA extraction with a lot of hands on effort. Instruments have been developed using microfluidics, nanotechnology and robotics to process the samples automatically. Automatic sample collection is being developed using remote access air-samplers to evaluate environments for high-risk pathogens of public health importance or routine sampling of animal environments, such as, poultry houses, swine barns, milking parlors, or food processing plants for specific pathogens of economic or public health importance.

One of the most important developments for the PCR technology was the discovery and use of the Taq DNA polymerase enzyme. By 1990, standard reagents (PCR buffer, dNTP, $MgCl_2$, and Taq DNA polymerase) were commercially available for PCR assays. One of the limiting factors at the time was the relatively small amount of sequence information for specific organisms. In 1990, we were attempting to develop species-specific primers for *Mycoplasma synoviae* and sequenced a portion of a DNA probe used in Israel to detect the organism. Specific primers were not developed using this approach. However, in 1991, the last half of the 16S rRNA gene sequence of *M. synoviae* was entered into the GenBank by an Australian team of scientists. A complete sequence of *Mycoplasma gallisepticum* 16 S rRNA gene sequence was found to perform a sequence alignment, which allowed us to select a set of species-specific primers for *M. synoviae*. The BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) came available and this was of great assistance in rapid computer evaluation of primer sequences for specificity by testing them against all sequences on the GenBank. As the years passed, sequence information concerning a greater number of organisms

were submitted to the various genetic libraries and thus we were able to develop a variety of primers and publish a PCR manual entitled “Nucleic acid amplification assays for diagnosis of animal diseases” (Lauerman, 1998). Recently PCR assays have been used to evaluate vaccines for the presence or absence of specific disease agents (Lauerman, 2002).

Improvements in buffers have given greater stability and longer activity for reagents and enzymes, which has contributed to improved PCR and RT-PCR techniques. Since the discovery of Taq DNA polymerase, many improvements have been made, such as, synthesis of a recombinant polymerase omitting undesirable side effects. Other polymerase enzymes have been isolated and evaluated from a number of thermophilic organisms having a variety of activities different from Taq DNA polymerase allowing for longer segments of DNA to be produced as PCR amplicon with greater accuracy.

New technology has been developed, such as, DNA microarray and DNA chips, that give hundreds to thousands more genetic information in a shorter period of time than the original PCR techniques. A detailed description of the DNA microarray procedure can be found on the following web sites http://www.vetscite.org/cgi-bin/pw.exe/issue3/000035/000035.htm or http://www.gene-chips.com/. The DNA microarray technology will also be described in detail in the next paper presentation at this conference.

The PCR technology has made it possible to genetically characterize microbial isolates inexpensively and rapidly for identification, typing, and epidemiological evaluation. The emphasis on Homeland Security and Bioterrorism Preparedness has expanded the need for more rapid and highly accurate diagnostic capabilities to protect the public health, animal agriculture and associated industries in the United States and the world.

References

1. Lauerman LH (1998). Nucleic acid amplification assays for diagnosis of animal diseases. Am Assoc Vet Lab Diagnosticians.
2. Lauerman LH (2002). Molecular techniques for evaluation of vaccines in response to national and international regulations. Proc US An Health Assoc, October 14, St Louis, MO.
3. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, and Erlich H (1986). Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. Cold Spring Harbor Symp Quant Biol 51:263-273.
4. Mullis K, and Faloona F (1987). Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol 155:335-350.