



**MVP Laboratories, Inc.**  
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# Research Roundup

## Welcome to the Inaugural Edition of Research Roundup!

MVP Laboratories strives to stay current in the latest developments in veterinary microbiology. In this quarterly publication, we will bring you topics of interest that may have application in your practice situation. Some of the research discussed here will be generated in our own laboratories and some will be summarized from recent journals. We will also occasionally address “frequently-asked-questions” that we get from our customers by phone or e-mail. We welcome ideas from you for future issues. Our contact information can be found on the back of this newsletter.

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## A Polymerase Chain Reaction (PCR) Primer

The molecular technique known as polymerase chain reaction, or PCR, is now used extensively in veterinary diagnostic medicine. Of the three most recent issues of *Journal of Veterinary Diagnostic Investigation* (Vol. 15, nos. 4,5,6) nearly half of the papers used PCR to identify and/or characterize animal pathogens. What exactly is PCR? The following synopsis written by Tabitha M. Towledge is from the Federation of American Societies for Experimental Biology website (<http://www.faseb.org/opa/bloodsupply/pcr.html>):

PCR requires a template molecule—the DNA or RNA you want to copy—and two primer molecules to get the copying process started. The primers are short chains of the four different chemical components that make up any strand of genetic material. These four components are like bricks or building blocks that are used to construct genetic molecules; in the lab they are called nucleotides or bases.

DNA itself is a chain of nucleotides. Under most conditions, DNA is double-stranded, consisting of two such nucleotide chains that wind around each other in the famous shape known as the double helix. Primers are single-stranded. They consist of a string of nucleotides in a specific order that will, under the right conditions, bind to a specific complementary sequence of nucleotides in another piece of single-stranded RNA or DNA.

For PCR, primers must be duplicates of nucleotide sequences on either side of the piece of DNA of interest, which means that the exact order of the primers' nucleotides must already be known. These flanking sequences can be constructed in the lab, or purchased from commercial suppliers.

There are three basic steps in PCR. First, the target genetic material must be denatured—that is, the strands of its helix must be unwound and separated—by heating to 90-96°C. The second step is hybridization or annealing, in which the primers bind to their complementary bases on the now single-stranded DNA. The third is DNA synthesis by a polymerase. Starting from the primer, the polymerase can read a template strand and match it with complementary nucleotides very quickly. The result is two new helices in place of the first, each composed of one of the original strands plus its newly assembled complementary strand.

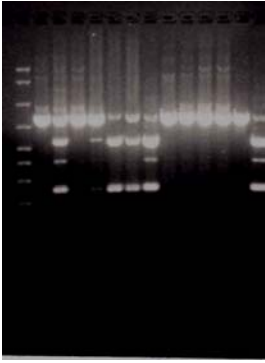
All PCR really requires in the way of equipment is a reaction tube, reagents, and a source of heat. But different temperatures are optimal for each of the three steps, so machines now control these temperature variations automatically.

To get more of the DNA you want, just repeat the process, beginning by denaturing the DNA you've already made. The amount will double every time. With the cycle of rapid heating and cooling controlled automatically, nature—aided by scientist-supplied primers, polymerase, nucleotides, and chemical reagents—does the rest. Each cycle takes only 1-3 minutes, so repeating the process for just 45 minutes can generate millions of copies of a specific DNA strand. Once the primers have been characterized and obtained, PCR can do in a week work that used to take a year.

### PCR requires:

- Target DNA
- Primers
- DNA polymerase
- Nucleotides
- Cycles of heating/cooling (thermocycler)

## Diagnostic Applications for PCR—Spotlight on *Haemophilus parasuis*



PCR-RFLP analysis of *H. parasuis* strains isolated from a large hog farm over a one-year period (B.C. Lin, American Association of Swine Veterinarians, March 2003)

As the preceding article mentions, PCR can generate millions of copies of genetic sequences, thereby producing enough nucleic acid material for further molecular characterization. One application is the use of PCR in differentiating strains of the same species of microorganism, or subtyping. This is important when trying to determine whether a disease outbreak was caused by a single strain or multiple strains of the same microorganism, especially where there are not reliable serotyping systems, or other means of subtyping. Such is the case with *Haemophilus parasuis*, an important pathogen in high-health status swine herds.

There are 15 serovars of *Haemophilus parasuis* described, as well as a high percentage of strains that do not serotype (1). Since little is known about the virulence factors of *H. parasuis*, and because there is poor correlation between virulence and serotype (2), serotyping alone does not always provide reliable information as to the clinical significance of isolates. Recently, a survey of 92 strains of *H. parasuis*, including 53 sero-nontypeable strains, was performed by using PCR-amplified sequences that were further characterized by restriction fragment length polymorphism (RFLP) (3). The resulting RFLP patterns could be divided into eight distinct groups, allowing an alternative way of differentiating sero-nontypeable strains. Further research is needed to discover the virulence factors and/or protective antigens necessary for vaccination that would be effective against all strains. In the meantime, a combination of serotyping and PCR-RFLP is used to select isolates of *H. parasuis* for autogenous bacterins.

### References:

1. Rapp-Gabrielson VJ and Gabrielson DA, 1992. Prevalence of *Haemophilus parasuis* serovars among isolates from swine. *Am J Vet Res* 53:659-664.
2. Rapp-Gabrielson VJ, Kocur GJ, Clark, JT, 1997. *Haemophilus parasuis*; Immunity in swine following vaccination. *Vet Med* 92:83-90.
3. Lin BC, 2003. Identification and differentiation of *Haemophilus parasuis* sero-nontypeable strains using a species-specific PCR and the digestion of PCR products with HindIII endonuclease.



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