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The Polymerase Chain Reaction (PCR) in the Diagnosis of Veterinary Infectious Diseases

By Vikram Misra, PhD

Effective management strategies for infectious diseases are predicated on accurate and specific identification of the infectious agent. Polymerase chain reaction (PCR) analysis has largely replaced more traditional approaches of antigen detection, microscopy and culture. This issue of *Large Animal Veterinary Rounds* discusses the use of PCR in the diagnosis of infectious disease. The review explains how PCR works, discusses applications in diagnostic medicine, and outlines the pitfalls.

Effective intervention in an infectious disease depends on the timely and accurate identification of the pathogen. Since disease-causing agents are often present in relatively small numbers, traditional techniques of identification relied on the biochemical or immunological agent characteristics that could only be applied after growing the agent. This is a time-consuming process; for some slow-growing organisms, laboratory results can take several weeks and the delay limits the value of the diagnostic information. In recent years, the emphasis of infectious-agent diagnosis has shifted from antigen detection by microscopy and culture, to molecular diagnostics. PCR is the most widely used of these new techniques and can be extremely sensitive, rapid, and relatively inexpensive. In some diagnostic laboratories, PCR has completely replaced traditional diagnostic techniques for organisms that are slow-growing, difficult to cultivate, or involve other time-consuming and expensive procedures. Although PCR does have limitations, these can be minimized in laboratories with standardized and strictly validated protocols, and high levels of quality control.

To enable the full use of PCR technology, veterinarians must understand the process sufficiently to use and interpret the results, and be aware of the limitations. Table 1 provides explanations for some PCR-related terms. Table 2 lists organisms for which PCR tests are available at the Prairie Diagnostic Services of the University of Saskatchewan. Other laboratories may offer these tests and additional ones.

PCR: the process

Musings on a late night drive along the California coastal highway resulted in the birth of PCR. Not only was this technique destined to become one of the most widely-used tools in molecular biology, it also earned Kary Mullis a Nobel Prize for Chemistry in 1993.¹

PCR is a method for producing an extremely large number of copies of a specific deoxyribonucleic acid (DNA) sequence. It is based on the simple principle that if you replicate (polymerize) 2 strands of DNA you get 4 strands. If you replicate them again you get 8 strands, and so on. If you form a chain of these reactions where they are consistently repeated, in < 20 cycles you have amplified your original 2 strands to more than a million. A million strands are much easier to detect than the original two strands.

The specific DNA sequences are amplified from a starting batch of DNA called a template. PCR can also detect ribonucleic acid (RNA) from RNA-containing viruses. However, RNA must first be converted to a DNA template using an enzyme called reverse transcriptase (RT). Therefore,



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| Table 1: Terms and definitions | |
|---|---|
| Terms | Definitions |
| Annealing (primers to template) | A step during a PCR cycle when the temperature is reduced to allow the primers to specifically bind to the template. |
| Ct (cycle threshold) | The cycle in real-time PCR when the signal crosses the threshold of detection – the cycle when the machine begins to detect the PCR product. This is an indication of the starting concentration of the template. The higher the starting concentration the lower the Ct. |
| Denaturing (template strands) | A step in a PCR cycle when the temperature is increased (usually to 95°-100°C) to separate the two complementary DNA strands. |
| Multiplex-PCR | A PCR reaction designed to detect > 1 target (> 1 pathogen or pathogen attribute) in a single reaction. |
| Primers | Short synthetic pieces of DNA that define the region to be amplified. Each primer is a perfect mirror image (complementary) to a portion of the DNA strand to be amplified (Figure 1). DNA polymerases must anchor to a primer before synthesizing new DNA. The primers therefore define where DNA synthesis begins and make the reaction specific. |
| Probes | Small synthetic pieces of DNA, similar to primers. While primers are used to amplify DNA, probes are designed to detect DNA and are often tagged with a radioactive or fluorescent molecule. |
| Real Time PCR (RT-PCR or qRT-PCR)* | PCR in which the amplified product fluoresces and is detected after each cycle by sensors in the thermocycler. The reaction can give quantitative information about the starting concentration of the template. The procedure can be used to quantitate the number of organisms/ viruses in a sample. In research laboratories, it is used to measure the level of expression of genes. |
| Reverse Transcriptase | Enzymes from retroviruses that convert RNA to DNA. Also called RNA-dependant DNA polymerases. |
| Reverse Transcriptase PCR (RT-PCR)* | PCR in which RNA is converted to DNA. A necessary step when using PCR to detect RNA viruses. |
| Sequence-based microbial species identification | A procedure based on amplifying variable regions of genes such as those of the 16s ribosomal subunit or Cpn60. Primers directed towards the conserved portions of these genes amplify intervening regions from all organisms. Determining the sequence of the PCR product and comparing it with those in databases then identifies the organism. |
| Taqman probes | One of the more common fluorescent probes used in real-time PCR. The Taq polymerase in the reaction activates the fluorescence in the probe as it degrades it. The process is reminiscent of the 1980s computer game “Pacman”. |
| Thermocycle | A sequence of changes in temperature: high temperature to denature the template, low temperature to allow annealing of primers to the separated template DNA strands followed by an increase in temperature to allow the thermostable DNA polymerase to replicate the template. A chain of these cycles or reactions makes up the PCR. |
| Thermocycler | A machine designed to rapidly heat and cool samples. In addition, a thermocycler for real-time PCR also detects the amplified PCR products as they are formed. |
| Thermostable DNA polymerase | DNA polymerases from organisms adapted to environments with high temperatures (undersea hot vents, hot springs, etc). The use of these enzymes allowed for the automation of PCR. The most commonly used thermostable DNA polymerase is Taq, from <i>Thermus aquaticus</i> , a bacterium isolated from hot springs in Yellowstone National Park. |

* In the literature, reverse transcriptase PCR and real time PCR are both called RT-PCR. This can be confusing.

this modification is called reverse transcriptase PCR (RT-PCR). In diagnostic laboratories, templates are extracted from clinical samples, often requiring nothing more than heating the sample to release DNA.

In addition to the template, 2 small pieces of synthetic DNA called primers are required. These primers are designed to define the region of DNA for detection and to ensure that only this region of the template is amplified. Primers can be designed to make PCR very specific; for instance, primers designed to amplify a gene from the bovine herpesvirus type 1 will only detect bovine herpesvirus type 1. Primers can also be designed to detect all

members of a family of organisms or viruses. A few years ago, primers designed to detect all herpesviruses were used to identify a previously unknown elephant herpesvirus, which was causing a high rate of neonatal mortality among zoo elephants.²

The specificity with which primers recognize their templates is based on the ability of a DNA sequence to bind faithfully to its “mirror image” (Figure 1). DNA is usually double stranded. The sequence of the four bases (adenine, cytosine, guanine, thymine), abbreviated A, C, G, and T on one strand, is mirrored exactly on the other strand. A on one strand is coupled to its complementary base T on the

Table 2: Some organisms or factors with available PCR

| |
|--|
| Bovine Coronavirus (respiratory) |
| Bovine Viral Diarrhea genotyping (BVD) |
| <i>Brachyspira (Serpulina) hyodysenteriae</i> , <i>B. innocens</i> and <i>B. pilosicoli</i> |
| <i>Chlamydia</i> species |
| <i>Chlamydia psittaci</i> |
| <i>Clostridium difficile</i> toxin typing |
| <i>Clostridium perfringens</i> toxin typing |
| <i>Coxiella burnetii</i> |
| <i>Cryptosporidium</i> species |
| <i>Elaphostrongylus</i> sp |
| <i>Parelaphostrongylus</i> sp |
| <i>Escherichia coli</i> 0157:H7 |
| <i>E. coli</i> virulence factor |
| Equine Influenza typing A1 (H7), A2 (H3) |
| Herpesvirus |
| Influenza A (all species) |
| Iridovirus |
| Lawsonia |
| Malignant Catarrhal Fever (MCF) (Ovine Herpesvirus-2) |
| <i>Mycobacterium avium</i> |
| <i>Mycobacterium bovis</i> |
| <i>Mycobacterium</i> ID by sequencing |
| <i>Mycobacterium</i> species |
| <i>Mycoplasma haemofelis</i> and <i>M. haemominutum</i> |
| <i>Mycobacterium paratuberculosis</i> (Johne's disease) |
| <i>Mycoplasma bovis</i> |
| <i>Mycoplasma haemolama</i> , <i>M. hemocanis</i> , <i>M. suis</i> , <i>M. wenyonii</i> |
| <i>Mycoplasma</i> species |
| <i>Mycoplasma hyopneumoniae</i> (Enzootic pneumonia) |
| <i>Mycoplasma gallisepticum</i> |
| <i>Mycoplasma synoviae</i> |
| <i>Pasteurella multocida</i> detection |
| <i>Pasteurella multocida</i> toxin gene |
| <i>Pasteurella multocida</i> toxin genotyping (PFLP) |
| <i>Pasteurella multocida</i> serogroup |
| <i>Pasteurella multocida</i> genotyping |
| PCR product sequencing |
| <i>Pneumocystis</i> species |
| Porcine circovirus-2S |
| PRRS virus (Porcine Reproductive Respiratory Syndrome) |
| PRRS typing |
| Rotavirus genotyping |
| <i>Salmonella</i> species |
| Swine Influenza typing H1, H3 |
| <i>Ureaplasma</i> |
| West Nile Virus (WNV) |

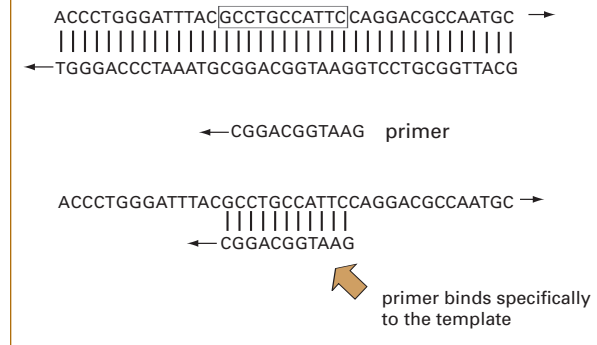
From Prairie Diagnostic Services: <http://www.usask.ca/pds/>

other strand, while G on one strand is coupled to its complementary base C. This complementarity of DNA allows the faithful replication of DNA prior to cell division. The DNA synthesizing machinery, while replicating one DNA strand, copies A for a T and G for a C. A primer designed to recognize a sequence on one strand of the template (Figure 1) will only bind to that sequence.

In addition to the template and primers, the building blocks for the new DNA molecules, the deoxynucleoside triphosphates (dNTPs), are also needed. A DNA polymerase, directed by the primers, copies the template into new DNA.

The reaction mixture is heated to between 95°C–100°C to break apart (denature) the two DNA strands of

Figure 1: Why primers bind specifically to their template DNA.



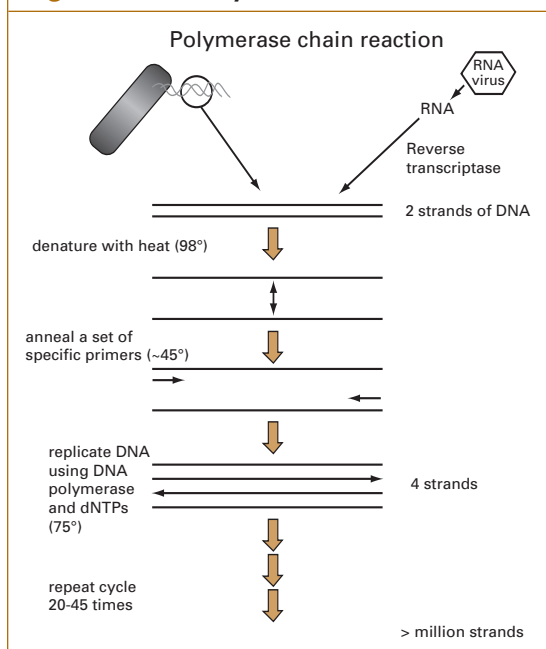
The sequence of two DNA strands is shown. The arrows represent the orientation of the sequence of bases in the two strands. The sequence is made up of the four bases, abbreviated **A**, **C**, **G**, and **T**; **A** in one strand couples with its complementary base **T** in the other strand and **C** in one strand couples with **G** in the other. A primer designed to recognize a sequence on the top strand (boxed) will only bind to that sequence.

the template. It is then cooled to allow the primers to anneal (bind specifically) to the separated template strands and to allow the polymerase to make more DNA. This cycle of heating and cooling is repeated 20 to 30 times (chain reaction). Theoretically, the amount of product doubles after each cycle (Figure 2). An excellent animation of the procedure can be seen at the web site of the Dolan DNA Learning Center.³

In the first article describing PCR, Saiki, Mullis, and co-workers used the DNA polymerase of *Escherichia coli* to amplify a portion of the gene for beta-globin.⁴ Since the high temperatures needed to denature DNA inactivate *E. coli* polymerase, the enzyme had to be added during each cycle. The use of thermostable DNA polymerases that can withstand temperatures of almost 100°C made it possible to automate the chain reaction, paving the way for widespread use of the technology. The most commonly used thermostable DNA polymerase is from the bacterium *Thermus aquaticus*. Thomas Brock discovered this organism in the 1960s in the hot springs of Yellowstone National Park. The polymerase, called Taq, can survive the repeated heating and cooling conditions of PCR and it replicates DNA at 74°C by catalyzing polymerization at a rate of 35–100 dNTPs per second. Thermostable DNA polymerases from other organisms, adapted for growth in extremely hot environments, can also be used.

With the primers as guides and dNTPs as building blocks, the DNA polymerase replicates a portion of the template, producing a product of a defined size. The product of the completed reaction is detected by electrophoresis through a gel and the subsequent staining of the DNA in the gel (Figure 3). A recent modification of PCR, called real-time PCR, has eliminated the need for electrophoresis. In real-time PCR, the amplified product fluoresces and is detected after each cycle in the chain reaction by a sensor in the PCR machine. Since it does not rely on electrophoresis

Figure 2: The Polymerase Chain Reaction.

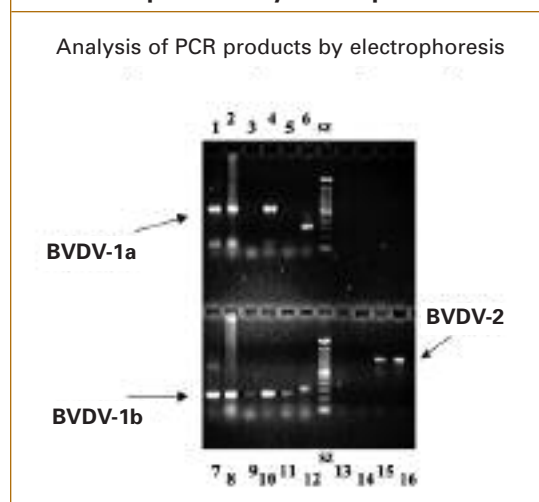


DNA from an organism is extracted. For RNA-containing viruses, the RNA must be converted to DNA with the reverse transcriptase enzyme. Heating to 98°C denatures the DNA (template). Short pieces of synthetic DNA, primers, are annealed to the template at a lower temperature (~ 45-55°C). The primers perfectly match the sequences that bracket the region to be amplified and only bind to the matching sequences in the template. Therefore, they define the portion of the DNA to be amplified and make the reaction specific. The temperature is increased to 75°C and a thermostable DNA polymerase in the reaction mixture then copies the portion of the template that lies between the primers. This process of denaturation, annealing, and polymerization is called a cycle. The cycle is repeated 20 to 40 times. At each cycle, the amount of amplified product doubles.

for detection of the product, real-time PCR is much faster than conventional PCR, with a turn-around time of a few hours. In addition to rapid analysis, real-time PCR also provides quantitative information on the amount of target DNA present in a sample. This is useful in instances where pathogen load (eg, the level of viremia in an animal) may have a predictive value for disease outcomes and it may also be useful in assessing the effects of treatment.

In addition, PCR can be set up to detect more than one pathogen or more than one attribute of a pathogen, in the same reaction. This process is called multiplex-PCR. For instance, while *Streptococcus suis* is widespread in pigs, some serotypes of this organism can cause severe disease and may even be responsible for disease in humans. PCRs can be set up that are designed not only to identify specific serotypes of *S. suis*, but also to detect virulence genes. Using this technique, Baums and others recently demonstrated that, while most wild boars in Germany harbour *S. suis*, 10% have serotypes and strains containing char-

Figure 3: Detection and analysis of PCR products by electrophoresis.



The figure shows the results of 16 serum samples (lanes 1-16) analysed by PCR using primers designed to detect bovine viral diarrhoea virus (BVDV) subtypes, 1a, 1b, or 2. The primers are designed to generate PCR products approximately 600, 300, and 1,000 base pairs in size, respectively. The products of PCR were electrophoresed on a gel and the gel stained for DNA. Lanes labelled "sz" contain size markers (100-1,500 base pairs), the lowest band is equivalent to 100 base pairs. The 600 base pair marker is brighter than the rest. Samples 1, 2, and 4 have a PCR product that is approximately 600 base pairs in size and, therefore, contains BVDV 1a. Samples 6, 7, 8, 9, 10, and 11 contain BVDV 1b (the product from sample 12 appears to be larger than the other BVDV 1b products and this could be considered a spurious result and reported as a "possible" BVDV 1b, until it can be confirmed by sequencing). Samples 15 and 16 contain BVDV 2. Sample 5 is negative and lanes 13 and 14 represent negative controls. (Courtesy of Prairie Diagnostic Services PCR Laboratory)

acteristics of bacteria isolated from cases of human meningitis.⁵

Real-time PCR can also be adapted for multiplex reactions. In this case, primer probes for different pathogens are coupled to chemicals that fluoresce at different wavelengths of light. A recent article described a real-time PCR reaction that can detect and distinguish between bovine viral diarrhoea (BVD) subtypes in one reaction.⁶ Real-time PCR machines are now available that can detect as many as 5 different fluorescent chemicals; therefore, they have the potential to test clinical samples for 5 different pathogens in a single reaction.

Noninfectious uses of PCR

PCR can detect genetic differences between animals and can serve as a useful tool for identifying desirable characteristics, such as disease resistance⁷ or meat quality. In such cases, the reactions are designed to distinguish between variants of genes that correlate either with susceptibility or resistance to disease, or

with desirable meat qualities. For instance, PCR has been used to identify genetic variations in the gene for calpastatin,^{8,9} a naturally occurring protein that inhibits normal tenderization of meat as it ages. Since some variants are associated with better meat characteristics, PCR tests to detect them can be useful in breeding programs intended to improve meat quality.

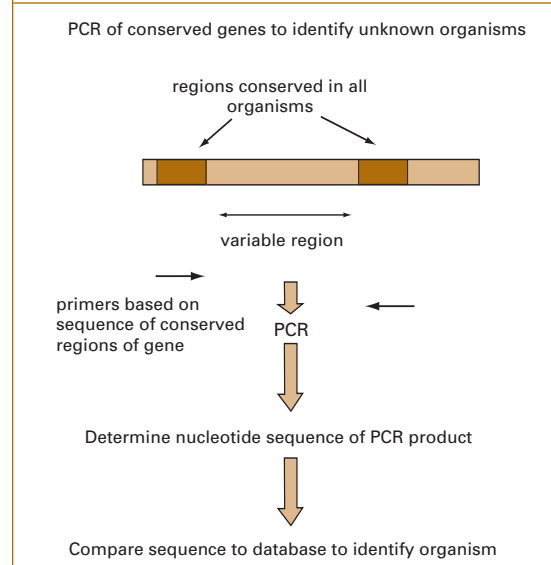
PCR limitations

Despite the obvious advantages of sensitivity, speed, and cost, PCR is not free of difficulties and limitations. Since the inception of PCR use in diagnostic medicine, one issue for laboratories has been the exquisite sensitivity of the reaction. This sensitivity often leads to false positives. Usually, the cause for false positives is contamination of test reactions by PCR products from previous reactions. Because there is an abundance of PCR product around during the gel electrophoresis step, contamination of the laboratory is inevitable. Most diagnostic laboratories now use protocols to minimize the possibility of contamination. These include physically separating the areas where reactions are assembled from areas where the completed reactions are analyzed, and incorporating modified dNTPs into the reaction that allow selective destruction of contaminating products in the PCR. Negative control reactions are also routinely included in the analysis. These control reactions contain everything included in test reactions, except for the template. An amplified product in the negative control warns of contamination and invalidates the test results.

PCR is also equally effective in detecting viable and inactivated organisms. Although potentially advantageous, because it extends the period for detecting an organism beyond the acute phase of the disease, it may also lead to confusing results in cases where a vaccine has been administered prior to the onset of disease.

Another limitation of conventional PCR is the reliance on primers that are based on sequence information from known organisms; as a result, PCR is unable to detect unexpected or unknown pathogens. The diagnostic laboratory must know which pathogens to expect before setting up a PCR reaction. A recent modification of the PCR can, to some extent, overcome this limitation. This modification, called sequence-based microbial species determination, relies on the observation that some genes, notably those of the 16s ribosomal RNA subunit in bacteria (18s in eukaryotes) and Chaperonin 60 (Cpn60) are conserved in all living organisms. Portions of these genes are almost identical in all species, while gene regions that lie between these conserved segments vary between species and subspecies. Therefore, the variable regions are more closely related when two species are closer to each other. The nucleotide sequences of ribosomal

Figure 4: Sequence-based identification of microorganisms using PCR of variable regions of ribosomal RNA and Cpn60 genes.



DNA is amplified using primers designed to recognize regions of the ribosomal RNA or Cpn60 genes that are conserved across species. The primers bind to the genes from all microorganisms and the PCR amplifies the variable regions that lie between the conserved portions of the genes. The sequence of the PCR product is then determined and compared with sequences in databases to identify the organism.

RNA and Cpn60 genes from thousands of organisms have been determined and are stored in freely accessible international databases. To identify an unknown organism, a PCR is conducted using primers representing the conserved regions (Figure 4). These primers amplify the intervening variable regions from all species. The nucleotide sequence of the amplified PCR product (variable region) is then determined and compared to variable region sequences in international databases. A match identifies a species. A close match indicates the species to which the unknown organism is most closely related. This technique can also be used to distinguish between closely related species. For instance, current methods for distinguishing between *Burkholderia pseudomallei* and *B. mallei*, the causative agents of melioidosis and glanders, respectively, rely upon an extensive set of biochemical reactions. Recently, Gee and others¹⁰ described a PCR technique for distinguishing between the two bacteria. The process relies on differences in the sequence of variable regions in the 16s RNA genes of the two bacteria. Similarly, Hill and others¹¹ have described a Cpn60 sequence-based technique for identifying *Campylobacter* species and distinguishing them from the closely related *Helicobacter* and *Arcobacter* species. While sequence-based techniques for identifying microorganisms are not yet available as routine tests, the automation of sequence determination and the reduc-

tion of costs are expected to make this a readily available diagnostic alternative in the near future.

Another limitation of PCR is that it requires the services of a diagnostic laboratory equipped with a thermocycler capable of rapidly increasing and decreasing the temperature of samples. While some semiportable units are now available, the "holy grail" for PCR-based applications would be the development of techniques making it possible to use PCR at "point of care" or "stall side." Recent developments by Piepenburg and others,¹² describing amplification reactions that can be performed at room temperature, are certainly steps to bring the goal closer.

Summary

PCR is a rapid, sensitive, and relatively inexpensive technology for identifying disease-causing microorganisms and viruses. PCR has limitations and pitfalls; however, most of these can be overcome by knowing how to interpret results generated by laboratories that practice high levels of quality control and use tests with properly validated techniques.

Dr. Vikram Misra, is Professor and Head of the Department of Veterinary Microbiology at the Western College of Veterinary Medicine. Dr. Misra's research is exploring the molecular basis for the pathogenesis of herpesvirus infections. His team has discovered unique proteins that regulate the response to stress in mammalian sensory neurons, and may play a role in herpesvirus latency and reactivation. At the University of Saskatchewan, Dr. Misra teaches undergraduate and graduate courses in Veterinary Virology, Advanced Virology, and Research Techniques in Molecular Biology.

References

1. Kary Mullis – Nobel Lecture, 2003 <http://www.nobel.se/chemistry/laureates/1993/mullis-lecture.html> Accessed: February 1, 2007.
2. Richman LK, Montali RJ, Garber RL, et al. Novel endotheliotropic herpesviruses fatal for Asian and African elephants. *Science* 1999;283(5405):1171-1176.
3. Dolan Learning Center, Cold Spring Harbor Laboratories. <http://www.geneticorigins.org/geneticorigins/pv92/pcr.html> Accessed: February 12, 2007.
4. Saiki RK, Scharf S, Faloona F et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985;230:1350-1354.
5. Baums CG, Verkuhlen GJ, Rehm T, et al. Prevalence of *Streptococcus suis* genotypes in wild boars of Northwestern Germany. *Appl Environ Microbiol* 2007;73(3):711-717.
6. Baxi M, McRae D, Baxi S, et al. A one-step multiplex real-time RT-PCR for detection and typing of bovine viral diarrhoea viruses. *Vet Microbiol* 2006;116(1-3):37-44.
7. Lillie BN, Hammermueller JD, Macinnes JI, et al. Porcine mannan-binding lectin A binds to *Actinobacillus suis* and *Haemophilus parasuis*. *Dev Comp Immunol* 2006;30:954-965.

8. Ciobanu DC, Bastiaansen JW, Lonergan SM, et al. New alleles in calpastatin gene are associated with meat quality traits in pigs. *J Anim Sci* 2004;82:2829-2839.
9. Schenkel FS, Miller SP, Jiang Z, et al. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Anim Sci* 2006;84:291-299.
10. Gee JE, Sacchi CT, Glass MB, et al. Use of 16S rRNA gene sequencing for rapid identification and differentiation of *Burkholderia pseudomallei* and *B. mallei*. *J Clin Microbiol* 2003;41(10):4647-4654.
11. Hill JE, Gottschalk M, Brousseau R, et al. Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that *Streptococcus suis* serotypes 32 and 34, isolated from pigs, are *Streptococcus orisratti*. *Vet Microbiol* 2005;107(1-2):63-69.
12. Piepenburg O, Williams CH, Stemple DL, Armes, NA. DNA detection using recombination proteins. *PLoS Biol* 2006;4:e204.

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