PCR clamping for selectively sequencing wild-type PRRSV in vaccinated herds

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Key points:

- Conventional ORF5 sequencing may not differentiate between wild-type or vaccine-like.
- Blocking the amplification of vaccine-like sequences it is possible to increase the likelihood of wild-type amplification.
- Clamping allows the amplification of the wild-type with mixtures containing as little as 10% of a mixture with the vaccine-like

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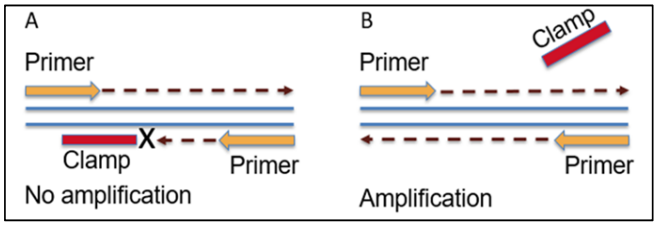
Transmission of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is frequently monitored via open reading frame 5 (ORF5) sequencing. This data is used to identify new introductions of the virus as well as to determine whether the virus is wild-type or vaccine-like. Since routine sequencing methods are often unable to differentiate wild-type from modified-live virus (MLV) PRRSV in vaccinated herds when both are present at the time of sampling, a tool which preferentially amplifies ORF5 of wild-type PRRSV in the presence of MLV vaccine virus would be highly beneficial.

"PCR clamping" technology has been described in which a modified oligonucleotide ("clamp") is used to block PCR amplification of a specific sequence[1,2]. Binding of the clamp to the DNA prevents PCR amplification and increases the likelihood of amplification of non-blocked sequences. Here we describe a PCR clamping assay to suppress ORF5 amplification of Ingelvac PRRS® MLV (I-MLV) vaccine to promote preferential amplification of wild-type PRRSV if also present in the sample.



For clamp design, PRRSV sequences from GenBank or generated from clinical cases at the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) were used to identify an I-MLV "signature sequence" present in sequences highly similar to I-MLV but not in wild-type PRRSV sequences. This oligonucleotide is included in the PCR reaction to block ORF5 amplification of I-MLV-like virus and enhances amplification of wild-type sequences in the sample. See Figure 1.

PRRSV PCR-positive samples previously characterized as "I-MLV vaccine-like" or "wild-type" based on ORF5 sequence, were sequenced with and without the clamp. Weaker positive samples (Ct >30) generally did not amplify regardless of whether or not the clamp was present. The remaining samples, all with ORF5 sequences 97 to 100% identical to I-MLV (N=30), resulted in a decrease or complete elimination of I-MLV ORF5 amplification. For the 22 samples < 91% identity to I-MLV, there was no discernable decrease in amplification with clamp compared to without clamp. Previously characterized virus isolates representing I-MLV-like and wild-type PRRSV were mixed in ratios of 10:0 to 0:10, respectively, with and without clamp and subsequently sequenced. Generally, without the clamp added, the level of wild-type virus that was needed to generate a wild-type sequence of high quality was a minimum of 70%. In contrast, when the clamp was added to the PCR reaction, high quality wild-type sequence could be obtained when the wild-type virus was present at as little as 10% of the mixture. See Figure 2 for sequencing data generated from a 50:50 mix of I-MLV and wild-type viruses.

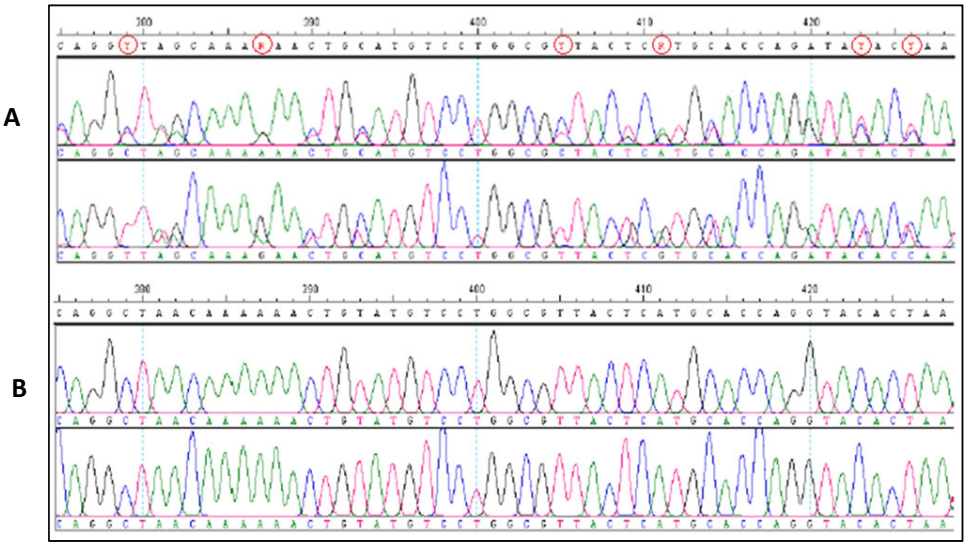


Figure 1 (top): Depiction of how the clamp prevents amplification (A) when a matching sequence is present in a sample, or has no effect (B) when a matching sequence is absent from the sample.

Figure 2 (Bottom): ORF5 sequencing data from a 50:50 mix of vaccine-like and wild-type PRRSV without (A) and with (B) clamp added to the PCR reaction. Note the ambiguous bases (double peaks with base designations circled in red), observed without clamp that are resolved when the clamp is included.

The clamp has been used on several ISUVDL cases in which original sequencing results identified I-MLV-like virus, and incorporation of the clamp also revealed the presence of a wild-type virus in the sample. "Wild-type" PRRSV sequencing is available at ISUVDL to preference wild-type sequencing over I-MLV vaccine, and the same technology is currently in development for the Zoetis Fostera® PRRSV vaccine.

References

1. Dabritz J et al. Detection of Ki-ras mutations in tissue and plasma samples of patients with pancreatic cancer using PNA-mediated PCR clamping and hybridization probes. British Journal of Cancer 2005; 92:405.
2. Orum H. PCR Clamping. Curr Issues Mol Biol. 2000; 2:27