NULLOMER EXTENSION BASED REAL-TIME PCR

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Polymerase chain reaction (PCR) is a conventional molecular biology technique which is widely used because of high sensitivity and rapid performance. In recent years, various modified versions of PCR like real-time PCR, qPCR, hot-start PCR and etc. are used for detection different microorganisms, including pathogenic ones. Real-time and quick PCRs are the most effective techniques for microbial diagnostics, as they use non-specific fluorescent dyes and fluorescently labeled probes, respectively, to visualize DNA fragments in real time.

Pasteurella multocida (*P. multocida*) infections pose significant challenges in clinical and veterinary settings. Here, we address these challenges by harnessing PCR techniques. Specifically, we adapted Allele-Specific Quantitative Extension PCR (ASQ-PCR) to target 5 *P. multocida* serogroups and *Kmt1* gene to develop a novel *Nullomer extension based Real-Time PCR approach*.

Firstly, nullomer oligonucleotides were synthesized using *ASM-800ET DNA/RNA Synthesizer by Biosset* in our laboratory. After, 7 different nullomers were tested for negative control with *human, Saccharomyces cerevisiae, Zea mays* DNA samples. The PCR results did not show any non-specific amplification with primer SPz3 with sequence 5'-CAGTCGGCTGAC-3'. This allowed us to proceed further experiments, such us positive control construction and synthesizing probes and primers for Real-Time intentions. The data from all available sequences of the serogroup-specific genes and Kmt 1 genes were analyzed using a multiple sequence alignment tool in MEGA11 software. The most conserved regions were selected as targets for further analysis.

To test a new real-time PCR assay, 6 different constructs of *P. multocida* were created, which contain 110-base pair target sequences from 5 different serogroups, as well as one construct that has a *Kmt1* gene target sequence, which is a marker for identifying *P. multocida*. For cloning purposes *pGEM®-T Easy Vector System by Promega* was used. Real-Time PCR was performed with recombinant Taq DNA polymerase produced in our laboratory. As a final step of research, PCR conditions, such as the concentrations of Mg^{2+}, dNTP, Taq DNA Polymerase, DNA template, primers and PCR program were optimized to obtain the best performance of Nullomer extension based Real-Time PCR.

Our study demonstrates the efficacy of these techniques in the detection of *P. multocida*, providing valuable tools for clinical and veterinary diagnostics. The ASQ-PCR modification enhances the specificity of detection, while the Nullomer extension based Real-Time PCR offers rapid and sensitive detection capabilities. These advancements contribute to the improvement of microbial diagnostics, particularly, in the context of *P. multocida* infections, facilitating timely intervention and management strategies. Furthermore, the development of pDNA controls ensures the reliability and reproducibility of testing procedures. Overall, our findings have implications for enhancing disease surveillance, control and prevention efforts in both human and animal populations.