

Designing Multiplex PCR Primers for the Simultaneous Detection of Three Respiratory Viruses Based on Fusion Protein Surface Antigen Gene

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Structured Abstract

Background: Respiratory viruses are major causes of illness and death worldwide. Due to overlapping symptoms, accurate and timely diagnosis is challenging for viruses such as *Metapneumovirus*, *Respirovirus*, and *Orthopneumovirus*. Traditional single-target diagnostic methods are often time-consuming and inefficient, highlighting the need for a rapid and multiplexed molecular approach. These three viruses have the Fusion (F) protein surface antigen gene that can be targeted for identification, since the F protein surface antigen gene is responsible for host cell entry. This study aims to design a set of multiplex PCR (mPCR) primers that can allow simultaneous detection of the three viruses with high specificity.

Methods: This study focused on designing mPCR primers targeting the F protein gene of the three viruses. 30 F protein DNA gene sequences of each virus were retrieved from GenBank and aligned using CLUSTAL Omega to identify conserved regions. Two forward primers and three reverse primers were designed based on alignment data. Primer properties such as GC content, melting temperature, and structural stability were evaluated using OligoCalc, while specificity was validated through BLAST analysis. A phylogenetic tree was constructed using MEGA11 software to highlight the specificity for each virus.

Results: Two forward primers named FA1 and FB1, and three reverse primers named RA1, RB1, and RC3, obtained were correspondent to each virus identification. The designed primers amplified distinct fragment sizes: 651 bp for *Metapneumovirus*, 998 bp for *Respirovirus*, and 1186 bp for *Orthopneumovirus*. BLAST analysis confirmed high specificity with no cross-reactivity. Phylogenetic analysis revealed clear separation among the three viral genera including the distinct branch distance from the outgroup *Alphainfluenzavirus*. These mPCR primers show strong potential for rapid, simultaneous detection of multiple respiratory viruses, supporting faster diagnosis and better outbreak management.

Conclusion: The findings of this study demonstrated the capability of the designed multiplex primers for the simultaneous detection of three common respiratory viruses. The BLAST analysis in GenBank database indicated that the primers have the capacity to precisely identify three virus targets.

Keywords: *Metapneumovirus*, *Respirovirus*, *Orthopneumovirus*, Multiplex PCR Primers and Fusion Protein

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