



COVID-19 target: A specific target for novel coronavirus detection

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ABSTRACT

An ongoing outbreak of pneumonia associated with a novel coronavirus has been reported worldwide and become a global health problem; hence, the diagnosis and differentiation of this virus from other types of coronavirus is essential to control of the disease. To this end, the analysis of genomics data plays a vital role in introducing a stronger target and consequently provides better results in laboratory examinations. The modified comparative genomics approach helps us to find novel specific targets by comparing two or more sequences on the nucleotide collection database. We, for the first time, detected ORF8 gene as a potential target for the detection of the novel coronavirus. Unlike previous reported genes (RdRP, E and N genes), ORF8 is entirely specific to the novel coronavirus (COVID-19) and has no cross-reactivity with other kinds of coronavirus. Accordingly, ORF8 gene can be used as an additional confirmatory assay.

1. Introduction

Coronaviruses are enveloped positive-sense RNA viruses belonging to the family Coronaviridae and the order Nidovirales spread among humans and animals (Richman et al., 2016). Although most of the coronavirus species (e.g., 229E, OC43, NL63, and HKU1) cause common cold in humans, some other species such as the Middle East respiratory syndrome coronavirus (MERS-CoV) and the severe acute respiratory syndrome coronavirus (SARS-CoV) cause severe respiratory diseases with mortality rates of 37% and 10%, respectively (MERS-CoV, W, n.d.; WHO, 2003). The World Health Organization (WHO) announced the outbreak of another coronavirus in China at the end of 2019 and named this novel coronavirus (COVID-19), which is currently a worldwide pandemic (WHO, 2020). The increasing cumulative incidence of different coronavirus genotypes throughout many countries poses a challenge to the public health laboratories in terms of diagnostic. Although molecular methods such as RT-PCR and real-time PCR are among the most common procedures in detecting coronavirus, the use of specific targets still is the first critical step in the accurate diagnosis of the agent. Bioinformatics analysis can assist in the identification of specific targets based on genetic diversity.

The present study aimed to introduce a novel specific target and evaluate the known target genes in order to analyze COVID-19

bioinformatically.

2. Methods

In this study, COVID-19 novel target was detected using the modified comparative genomic analysis (Kakhki et al., 2020; Kakhki et al., 2019; Neshani et al., 2018). The genome of Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1 (NC_045512) was considered as reference strain and compared with the other types of coronavirus isolates. The gene exhibiting less cross-reaction with the other coronaviruses was considered as the conserved sequences of COVID-19. Then the primers and a probe were designed by Primer Premier Software version 5.0 (Premier Biosoft Intl., CA USA), and then the secondary structures and the predicted melting temperature were checked. Afterwards, the specificity of the primers was determined bioinformatically using BLAST software for all databases to check any cross-reactivity with other bacterial or human genomes.

Furthermore, we analyzed the targets, primers, and probes, which were introduced previously (Corman et al., 2020b) for the detection of the novel coronavirus using the Basic Local Alignment Search Tool (BLAST) search.

Abbreviations: COVID-19, coronavirus disease 2019; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; ORF-8, open reading frame 8; BLAST, basic local alignment search tool; RT-PCR, reverse transcription polymerase chain reaction

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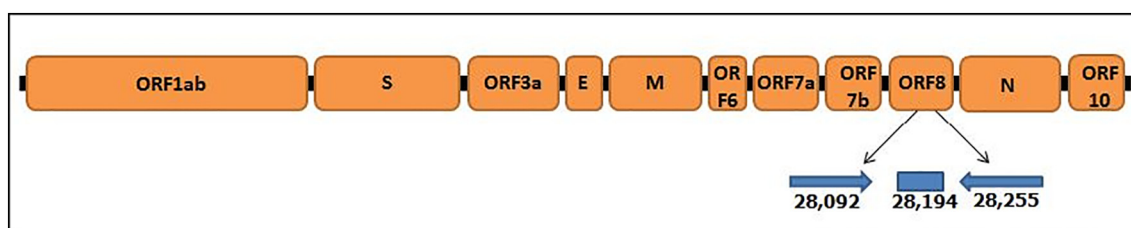


Fig. 1. The positions of ORF8 target, primers and probe on Wuhan-CoV genome.

Table 1

Sequences and other character of designed primers and probe in this study.

Assay/use	Oligonucleotides	Sequence (5' - 3')	Length	Tm	GC%	product length
ORF8	K_COV-F1	TCTAAATCACCCATTAGTACATC	24	56.26	37.50	164 bp
	K_COV-R1	ATGAAATCTAAAACAACACGAACG	24	56.79	33.33	
	K_COV-P1	CGTTGTTCTGTTCTATGARGACTT	23	57.18	39.13	-

Table 2

Reported probes for detection of COVID-19 (Corman et al., 2020a; Corman et al., 2020b).

Assay/use	Oligonucleotide ID	Sequence (5'-3')
RdRP gene	RdRP_SARSR-P2	CAGGTGGAACCTCATCAGGAGATGC
	RdRP_SARSR-P1	CCAGGTGGWACRTCATCMGGTGATGC
E gene	E_Sarbeco_P1	ACACTAGCCATCCTTACTGCGCTTCG
N gene	N_Sarbeco_P1	ACTTCCTCAAGGAACAACATTGCCA

3. Results

According to our survey, ORF8 gene, for the first time, was recognized as a potential target to detect novel coronavirus. The designed primers (K_COV-F1 and K_COV-R1) identified complete genomes of COVID-19 under different names as Severe Acute Respiratory Syndrome Coronavirus 2, Wuhan Seafood Market Pneumonia Virus, Bat Coronavirus and Pangolin Coronavirus, as published in 2019 and 2020 using Primer-BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). We also designed a specific probe (K_COV-P1) for the novel Coronavirus to differentiate COVID-19 from all other human coronaviruses. The location and characteristics of the designed primers and probe are illustrated in Fig. 1 and Table 1.

In 2020, Corman et al. (2020b) reported RdRP, E and N genes for the detection of the novel coronavirus (Table 2). The bioinformatic analysis of the probes designed to identify the novel coronavirus was evaluated by BLAST search. The designed probe located in N gene (N_Sarbeco_P1) illustrated a lot of cross-reactions with Coronavirus BtRs-BetaCoV (MK211374- MK211378), SARS coronavirus Urbani (MK062179-MK062184), Bat coronavirus (KY770858-KY770859), SARS coronavirus (AH013708-AH013709), and others. The designed probe located in E gene (E_Sarbeco_P1) also indicated some cross-reactions with Coronavirus BtRs-BetaCoV (MK211374- MK211378), SARS Coronavirus Urbani (MK062179-MK062184), Bat SARS-Like Coronavirus (KY417142-KY417152), Bat Coronavirus (KY938558), and many others. Two designed probes located in RdRP gene were also appraised. The first one (RdRP_SARSR-P1) covers many coronavirus isolates, including Bat SARS-like Coronavirus (MG772904-MG772932), *Rhinolophus pusillus* Coronavirus (KY775091), Bat SARS-like Coronavirus (MG772903) and many others; because degenerate bases like W, R, and M nucleotide codes were used to design probes. The second probe (RdRP_SARSR-P2) was more specific for COVID-19 and could not detect the other human coronaviruses, with the exception of *Rhinolophus* Bat Coronavirus BtCoV (KP876546.1), as reported in 2016. All these cross-reactions are associated with the sequences, as mentioned in papers published in 2018 and before.

4. Discussion

The Different types of RNA viruses have been able to cause major human concerns in health fields regarding the epidemics transmission (Keikha et al., n.d.). So, specific identification of the causative agent could be crucial for disease prevention.

Despite the discovery of some targets for the detection of the novel coronavirus, the specific target located in another gene could provide better results in laboratory examinations. ORF8 gene is a new target not being addressed before. This gene contains highly specific regions for COVID-19, which makes it suitable for primer and probe design. ORF8 gene has also common regions with Bat SARS-like Coronaviruses (MG772934.1 and MG772933.1) and is differentiated from COVID-19 using a specific probe (K_COV-P1).

The WHO recommends RdRP, E and N genes for the detection of the novel coronavirus (Corman et al., 2020a): E gene for first line screening, RdRp gene for confirmatory assay, and N gene for additional confirmatory assay. Although these genes reported as potential targets for the detection of coronavirus, we found out that only one of them (RdRP_SARSR-P2) was almost specific for the new coronavirus and the other introduced probes would detect the other types of coronaviruses. In this regard, the false-positive test results may extend for COVID-19, and many patients with mild symptoms may be infected by the other types of coronavirus. Accordingly, introducing another target such as ORF8 and designing specific primers and probes for the detection of the novel coronavirus would be useful for additional confirmatory assay. This study was conducted bioinformatically, and laboratory examinations are needed to confirm ORF8 gene as a potential target using RT-PCR, Real time PCR, or Line probe assay (Aryan et al., 2020).

Transparency document

The Transparency document associated with this article can be found, in online version.

Declaration of competing interest

The authors declare that they have no competing interests.

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