

# Mesoscopic evaluation of DNA mismatches in PCR primers for SARS-CoV-2 detection

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## Abstract

The pandemic of COVID-19 brought the necessity to a large testing in population. As a golden standard for molecular tests, techniques based in PCR (Polymerase Chain Reaction) has been used to detect SARS-CoV-2 virus, such as RT-PCR (reverse transcription PCR). For the amplification of viral target is used a set of primers to hybrid with. These oligos are single strands DNA sequences of 18-20 bases in length and are designed for sense and antisense direction. An important parameter to obtain a good performance of primers is the melting temperature which is related to the efficiency of primer to hybridise to the DNA molecule. Primers are designed to bind complementarily to DNA, however, it may be include single mismatches in the hybridisation. Mismatch presence can influence in the stabilisation of DNA molecule. In the case of PCR process, one or more mismatches can change the melting temperature of primers and may be interfere in the amplification of DNA molecule. Focusing in how mismatches may impact the detection of SARS-CoV-2 by PCR techniques, we collected and analysed 19 PCR primers sets to verify the behaviour of their melting temperatures in presence of up to three consecutive mismatches. We aligned the primers sets with 21665 genomes of SARS-CoV-2 and applied the Peyrard-Bishop mesoscopic model to obtain the melting temperatures for the resulted alignments. We compared the calculated melting temperatures for mismatch and perfect alignments. Furthermore, we collected genomes of SARS-CoV and other five coronaviruses to be our control group performing the same workflow. In addition, we collected some data that can contribute to an optimization of primers sets for PCR diagnostic method for SARS-CoV-2. Our results indicate numerous instances where the mismatch presence does not destabilize the primers ensuring their detection capacity.

Funding:

Link to Video: