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Nucleotide Sequence Sharing between the Human Genome and Primers for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Detection

Darja Kanduc[1]

[1] Department of Biosciences, Biotechnologies, and Biopharmaceutics, University of Bari, Bari, Italy

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Address for correspondence Darja Kanduc, PhD, Department of Biosciences, Biotechnologies, and Biopharmaceutics, University of Bari, Via Orabona 4, Bari 70125, Italy (e-mail: dkanduc@gmail.com).

Abstract

This study shows that oligonucleotide sequences are shared between the human genome and primers that have been proposed/used for SARS-CoV-2 detection by polymerase chain reaction (PCR). The high level of sharing (namely, up to 19mer with a maximum number of gaps equal to 2) might bear implications for the diagnostic validity of SARS-CoV-2 detection by PCR.

Keywords

- PCR primers
- SARS-CoV-2 detection
- false positives

Introduction

Defining the relationship(s) between infectious agents and the human host is a crucial topic in immunology, microbiology, and infectious medicine. Although it has been proposed that genetic factors might play a role,[1,2] the exact mechanisms of chronic infections and occasional (re)activation of pathogens in the human host are largely misunderstood and poorly studied. The issue became even more relevant in light of the recent Ebola virus, Dengue virus, and SARS outbreaks associated with high morbidity and mortality.[3–5] In this context, there is a need not only for knowing the molecular basis of infections to define effective and safe preventive and therapeutic interventions but also for sensitive and specific diagnostic tools. Indeed, accurate screening of asymptomatic, presymptomatic, and symptomatic subjects might be key to effective epidemiological measures during pandemics. However, especially in analyzing SARS-CoV-2 as a paradigmatic example, contrasting data have been reported on the analytical performance of SARS-CoV-2 detection methods and claims about the rates of false negatives and false positives have been published.[6–11]

On the basis of all these, this study focused on the possible genetic basis of potential false polymerase chain reaction (PCR) results by comparing the nucleotide sequence of proposed/used SARS-CoV-2 primers versus the human genome. The scientific rationale is that—given the high level of amino acid sequence sharing between SARS-CoV-2 proteins and the human proteome[12–15]—parallel sequence matching at the nucleotide level might exist between the SARS-CoV-2 primer sequences and the human genome, in this way possibly explaining the generation of false-positive SARS-CoV-2 detection results. Data are reported here that confirm the likelihood of the research hypothesis.

De facto, using the nucleotide Basic Local Alignment Search Tool (BLASTn) program from NCBI (http://blast.ncbi.nlm.nih.gov,[16,17] a sample of 12 primers retrieved from literature,[18,19] proposed/used even by government health institutions[19] to detect SARS-CoV-2, and described here in ►**Table 1**, was analyzed for nucleotide sequence sharing with the human genome. BLASTn analyses documented a relevant viral versus human oligonucleotide overlap, with shared primer sequences repeatedly present in the human genome, disseminated among different chromosomes, and located in plus strands, minus strands, mRNAs, pseudogenes, etc. Due to space constraints, an *in extenso* description of the complete nucleotide sequence sharing is practically not possible, and only a synthetic snapshot is shown in ►**Table 2**.

In conclusion, this communication highlights the likelihood that viral versus human nucleotide sequence overlap

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Table 1 Nucleotide sequence of primers used/proposed for PCR detection of SARS-CoV-2[a]

Primer no.	Target gene[b]	Primer direction	Primer nucleotide sequence
1	S 2	F	CCACTAGTCTCTAGTCAGTGTGTTAAT
2	S 2	R	AAACTGAGGATCTGAAAACTTTGTC
3	8	F	GGAGCTAGAAAATCAGCACCTTTAA
4	8	R	TCGATGTACTGAATGGGTGATTTAG
5	E	F	ACAGGTACGTTAATAGTTAATAGCGT
6	E	R	ATATTGCAGCAGTACGCACAGA
7	N	F	GACCCCAAAATCAGCGAAAT
8	N	R	TCTGGTTACTGCCAGTTGAATCTG
9	N	F	GGGGAACTTCTCCTGCTAGAAT
10	N	R	CAGACATTTTGCTCTCAAGCTG
11	N	R	TAATCAGACAAGGAACTGATTA
12	N	F	TGGCAGCTGTGTAGGTCAAC

Abbreviations: F, forward; PCR, polymerase chain reaction; R, reverse.
[a]Primers retrieved from Gadkar et al[18] and Qasem et al,[19] and further details and references therein.
[b]Gene names given according to Uniprot.[20]

Table 2 Oligonucleotide sharing between the human genome and polymerase chain reaction (PCR) primers proposed/used to detect SARS-CoV-2: a few examples[a]

1. CCACTAGTCTCTAGTCAGTGTGTTAAT

   Glypican 5 (GPC5), Chromosome 13, Strand: Plus/Plus

   864805 TCTAGTCAGTGTGTTAAT 864822

2. AAACTGAGGATCTGAAAACTTTGTC

   DEP domain containing 5, Chromosome 22, Strand: Plus/Minus

   132374 CTGAGGATCTGAAAACTTT 132356

3. GGAGCTAGAAAATCAGCACCTTTAA

   DNA damage regulated autophagy modulator 2 (DRAM2), Chromosome 1, Strand: Plus/Plus

   3702 AGAACATCAGCACCTTTAA 3720

4. TCGATGTACTGAATGGGTGATTTAG

   Isolate CHM13 chromosome 17, Strand: Plus/Plus

   5169199 GATGTACTGAAAGGCTGATTTA 5169220

5. ACAGGTACGTTAATAGTTAATAGCGT

   Chromosome 18, SeqID:AP023478.1,Strand: Plus/Minus

   34259565 GTACGTTAATAGTAAATA 34259548

6. ATATTGCAGCAGTACGCACAGA

   Hemicentin 1, HMCN1, Chromosome 1, Strand: Plus/Plus

   379167 ATTGCAGCAGTAAGCACAG 379185

7. GACCCCAAAATCAGCGAAAT

   SLAM family member 8, SLAMF8, transcript variant 2, mRNA, SeqID: NM_001330741.2, Strand: Plus/Plus

   161 CCCCAACATCAGCGAAAT 178

8. TCTGGTTACTGCCAGTTGAATCTG

   Sciatic injury induced incRNA upregulator of SOX11, long non-coding RNA, SequID: NR_026832.1, Strand: Plus/Minus

   9779 TGGTTACTCCCAGTTGAAT 9761

9. GGGGAACTTCTCCTGCTAGAAT

CHM13 chromosome 20, SeqID: CP068258.2 Strand: Plus/Plus

11065407 AACTTCTCCAGCTAGAAT 11065424

10. CAGACATTTTGCTCTCAAGCTG

Rho GTPase activating protein 6, ARHGAP6), RefSeqGene on chromosome X, SeqID: NG_012494.2,Strand: Plus/Minus

488854 CAGACATTTTGCTTTCAAG 488836

11. TAATCAGACAAGGAACTGATTA

Chromosome 3 clone RP11-24C3, SeqID: AC104448.2, Strand: Plus/Minus

13048 TAATCAGACAAGGCACTGA 13030

12. TGGCAGCTGTGTAGGTCAAC

BAC clone RP11-150O15, SeqID: AC020591.7, Strand: Plus/Plus

24820 TGGCTGCTGTGTAGGTCAA 24838

[a]Twelve primers described in ►**Table 1** and derived from Gadkar et al[18] and Qasem et al[19] were analyzed for sharing of nucleotide sequences with the human genome. BLASTn[16,17] was used to find and localize regions of identity in the human nucleotide collection covering genomic and transcript sequences; further details are available at http://blast.ncbi.nlm.nih.gov. The 12 primers are listed with shared nucleotide sequences underlined.

can interfere with nucleic acid amplification testing and generate PCR false-positive results in SARS-CoV-2 detection, in this way affecting medical diagnoses.

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None.

Conflict of Interest
None declared.

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