



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

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Glob Med Genet 2022;9:182–184.

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Abstract

Keywords

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

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.

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 institutions¹⁹ 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

received
November 29, 2021
accepted
December 29, 2021

DOI <https://doi.org/10.1055/s-0042-1743260>.
ISSN 2699-9404.

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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	AAACTGAGGATCTGAAAACCTTTGTC
3	8	F	GGAGCTAGAAAATCAGCACCTTTAA
4	8	R	TCGATGTAATGAATGGGTGATTAG
5	E	F	ACAGGTACGTTAATAGTTAATAGCGT
6	E	R	ATATTGCAGCAGTACGCACAGA
7	N	F	GACCCCAAAATCAGCGAAAT
8	N	R	TCTGGTACTGCCAGTTGAATCTG
9	N	F	GGGGAACCTTCTCCTGCTAGAAT
10	N	R	CAGACATTTTGTCTCAAGCTG
11	N	R	TAATCAGACAAGGAAGTATTA
12	N	F	TGGCAGCTGTGTAGGTCAAC

Abbreviations: F, forward; PCR, polymerase chain reaction; R, reverse.

^aPrimers retrieved from Gadkar et al.¹⁸ and Qasem et al.¹⁹ and further details and references therein.

^bGene 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. AAACTGAGGATCTGAAAACCTTTGTC
DEP domain containing 5, Chromosome 22, Strand: Plus/Minus
132374 CTGAGGATCTGAAAACCTT 132356
3. GGAGCTAGAAAATCAGCACCTTTAA
DNA damage regulated autophagy modulator 2 (DRAM2),
Chromosome 1, Strand: Plus/Plus
3702 AGAACATCAGCACCTTTAA 3720
4. TCGATGTAATGAATGGGTGATTAG
Isolate CHM13 chromosome 17, Strand: Plus/Plus
5169199 GATGTACTGAAAGCTGATTAA 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 ATTCAGCAGTAAGCACAG 379185
7. GACCCCAAAATCAGCGAAAT
SLAM family member 8, SLAMF8, transcript variant 2, mRNA,
SeqID: NM_001330741.2, Strand: Plus/Plus
161 CCCAACATCAGCGAAAT 178
8. TCTGGTACTGCCAGTTGAATCTG
Sciatic injury induced incRNA upregulator of SOX11, long
non-coding RNA, SequID: NR_026832.1, Strand: Plus/Minus
9779 TGGTACTCCAGTTGAAT 9761

9. GGGGAACTTCTCCTGCTAGAAT

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

11065407 AACTTCTCCAGCTAGAAT 1106542410. 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 RP11-150015, SeqID: AC020591.7, Strand: Plus/Plus

24820 TGGCTGCTGTGTAGGTCAA 24838

^aTwelve primers described in ► **Table 1** and derived from Gadkar et al¹⁸ and Qasem et al¹⁹ 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.

Funding

None.

Conflict of Interest

None declared.

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