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

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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

Table 1

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer 1</td>
<td>AGCTGCAATCAGTGGGACTC</td>
<td>16</td>
</tr>
<tr>
<td>Primer 2</td>
<td>GTCAGTGGCAGTCGTACG</td>
<td>17</td>
</tr>
</tbody>
</table>

De facto, using the nucleotide Basic Local Alignment Search Tool (BLASTn) program from NCBI,6 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 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 proteome12–15—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.

Keywords

- SARS-CoV-2 detection
- false positives

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

<table>
<thead>
<tr>
<th>Primer no.</th>
<th>Target gene</th>
<th>Primer direction</th>
<th>Primer nucleotide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S 2</td>
<td>F</td>
<td>CCACCTAGTCTCTAGTGCGATGTTAAT</td>
</tr>
<tr>
<td>2</td>
<td>S 2</td>
<td>R</td>
<td>AAGACTGAGATCCTGAAAAACCTTTGTC</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>F</td>
<td>GGGGTAAACTGCACTGGATGTTAAT</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>R</td>
<td>TCCAGTGCTGAATGGGTAAT</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>F</td>
<td>ACACGAGTTGAATTATGTTGAATGGCT</td>
</tr>
<tr>
<td>6</td>
<td>E</td>
<td>R</td>
<td>ATATTGCCAGCGAGACCGACAGA</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>F</td>
<td>GACCCAAAATCCGGCGAAT</td>
</tr>
<tr>
<td>8</td>
<td>N</td>
<td>R</td>
<td>TCCTGTTAAGCTGGATGAATCTG</td>
</tr>
<tr>
<td>9</td>
<td>N</td>
<td>F</td>
<td>GGAGAACTTTCTGCTGCTCTAA</td>
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<tr>
<td>10</td>
<td>N</td>
<td>R</td>
<td>CAGACATTGCTCTCAAGCTG</td>
</tr>
<tr>
<td>11</td>
<td>N</td>
<td>R</td>
<td>TAATCAGACAGAAACTGATTA</td>
</tr>
<tr>
<td>12</td>
<td>N</td>
<td>F</td>
<td>TGGCAGCTGTGTTAGGTCAAC</td>
</tr>
</tbody>
</table>

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

*Primers retrieved from Gadkar et al18 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

1. CCACCTAGCTCTTAGCAGGTTAAT
   Glypican 5 (GPC5), Chromosome 13, Strand: Plus/Plus
   684805 TCTAGCTAGTGTTAAT 684822
2. AAAGCTGAGATCCTGAAAAACCTTTGTC
   DEP domain containing 5, Chromosome 22, Strand: Plus/Minus
   132374 CTGAGCTCGCGAAAACTTTA 132356
3. GGAGCTGAGATCCTGAAAAACCTTTA
   DNA damage regulated autophagy modulator 2 (DRAM2), Chromosome 1, Strand: Plus/Plus
   3702 AGAACATCAGACACTTGA 3720
4. TCAGATGTACTGAGAGGTGCTGGTAG
   Isolate CHM13 chromosome 17, Strand: Plus/Plus
   5169199 GATGTACTGAAAGGGCTGATT 5169220
5. ACAGCTGAGATCCTGAAAAACCTTTA
   Chromosome 18, SeqID: AP002347.1, Strand: Plus/Minus
   3429565 CTGCTTAAATAGTATAATA 3429548
6. ATATTGCCAGCGAGACCGACAGA
   Hemocytin 1, HMCN1, Chromosome 1, Strand: Plus/Plus
   379167 ATTTGCAGCTGCAAGCAG 379185
7. GCACCTAGCTCTTAGCAGGTTAAT
   SLAM family member 8, SLAMF8, transcript variant 2, mRNA, SeqID: NM_001330741.2, Strand: Plus/Plus
   161 CCACCTAGCAGGTTAAT 178
8. TCTAGCTAGTGTTAATGGAGT
   Sciatic injury induced incRNA upregulator of SOX11, long non-coding RNA, SeqID: NR_026832.1, Strand: Plus/Minus
   9779 TGTTAATCCCGAGTTGAT 9761
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.

References

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