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Original Article

Quantitative Analysis of Nucleic Acid Content in Spikevax (Moderna) and BNT162b2 (Pfizer) COVID-19 Vaccine Lots

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Abstract

Background: The emergence of mRNA-based COVID-19 vaccines represented a significant advancement in public health response during the pandemic. However, questions have arisen regarding the consistency of their nucleic acid content, the presence of undeclared genomic material, and the continued use of outdated viral sequences.

Methods: In this study, 17 lots of Spikevax (Moderna) and 7 lots of BNT162b2 (Pfizer) vaccines were analyzed for nucleic acid content using multiplex quantitative Real-Time PCR. Samples were assessed for mRNA identity, quantity, homogeneity, and the presence of undeclared nucleic acids, including DNA elements. The stability of nucleic acid content in expired vaccine lots stored at -80°C was also examined.

Results: Quantitative analysis confirmed the presence of mRNA sequences consistent with the Spikevax and BNT162b2 vaccines. However, variations in nucleic acid quantity were observed across lots. DNA sequences, including Escherichia coli genomic fragments, were detected in some samples. Despite the evolution of circulating SARS-CoV-2 variants, both vaccine types retained the original "Wuhan" S protein sequence. Expired vaccine samples exhibited reduced nucleic acid integrity. No evidence of SV40 was identified.

Conclusion: The presence of undeclared DNA sequences and variability in nucleic acid content across lots underscores the need for enhanced quality control in vaccine manufacturing. The presence also raises InflammoThrombotic Immunologic Response (ITIR) concerns. Regulatory oversight should address the potential risks associated with genetic material inconsistencies to ensure vaccine safety and efficacy.

Keywords: COVID-19 vaccine; mRNA; Nucleic acid

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Introduction

Infectious processes, including that caused by Severe Acute Respiratory Syndrome Coronavirus number 2 (SARS-CoV-2) result in an InflammoThrombotic Immunologic Response (ITIR) Disease (ITIRD) [1,2], in this instance Coronavirus Disease first described in 2019 (COVID-19). While medical management is possible [3], enhancement of immunity can be acquired through the administration of vaccines [4,5].

The development of mRNA-based COVID-19 vaccines, such as Spikevax (Moderna) and BNT162b2 (Pfizer), has been pivotal in mitigating the global pandemic. These vaccines rely on modified messenger RNA (mRNA) molecules to instruct cells to produce the SARS-CoV-2 spike (S) protein, triggering an immune response.

While mRNA vaccines underwent rigorous clinical evaluation, emerging concerns have prompted further examination of their nucleic acid content and batch consistency. In particular, discrepancies in genomic material, including the presence of undeclared DNA sequences, have raised questions about manufacturing integrity and potential biological risks.

This study was conducted in response to a formal request by Peter Kotlár, MD, Plenipotentiary for the Government of the Slovak Republic, to assess the nucleic acid content in multiple lots of Spikevax and BNT162b2 vaccines. The analysis aimed to:

- Identify the nucleic acids present in the vaccine preparations.
- Quantify nucleic acid content across multiple lots.
- Evaluate batch homogeneity.
- Determine whether undeclared genetic material was present.
- Assess potential biological implications of unexpected nucleic acids.
- Examine the effects of expired storage conditions on nucleic acid stability.
- The findings provide insights into vaccine composition, stability, and manufacturing practices, with implications for public health policy and regulatory oversight.

Methods

Terminology

Given the relative infancy of this field of genetic vaccines, we provide the following terminology.

mRNA – messenger RNA; a nucleic acid to convey the genetic information contained in it into a protein by translation, DNA – deoxyribonucleic acid, in this project used in the sense of double-stranded DNA (dsDNA), Multiplex quantitative Real-Time PCR – Real-time PCR, allowing quantitative simultaneous detection of several molecular targets at the same time, Expression cloning vector – a circular double-stranded DNA molecule, which is used for subcloning, propagation and expression of a transgene. Transgene – a foreign, often artificially created, synthetic nucleic acid construct (typically DNA) intended for the purposes of genetic modification of a recipient cell or organism.

GMO – genetically modified organism, and S protein – one of the SARS-CoV-2 proteins composing the viral spike which attaches to the surface of cells, the genetic sequence of which differs between individual variant strains of the virus.

Sample collection and handling

Twenty-four distinct vaccine lots (17 Spikevax, 7 BNT162b2) were provided under controlled conditions. Each lot contained 10 original, unopened vials stored at -80°C. Transport conditions were verified using an onboard thermometer to ensure continuous temperature control.

The package contained the following lots

Spikevax (Moderna): MV1013A, 200023A, 200156A, 223049, 200090A, 200106A, 200100A, 3005885, 3005836, 000090A, 000058A, 3005241, 3005697, 3006272, MV1018A, 400012A, 400011A.

BNT162b2 (Pfizer): FP9632, 1F1051A, 1LO84A, 1F1047A, 1F1059A, 1F1055A, PCB0020.

Nucleic acid isolation

Samples were thawed rapidly and processed using the QIAamp DNA Mini Kit (Qiagen, DE) following the manufacturer's protocol. DNA and RNA were eluted in 50 µl of elution buffer. Reverse transcription was performed using the verso cDNA Kit (ThermoFisher Scientific, USA) at 47°C for 1 hour. All isolates were subsequently stored at -80°C.

Analytical procedure used

From each tested lot (containing 10 individual vials in labeled boxes), 5 original, unopened, unused vials of the product were used. The remaining unused vials were left intact as reference material at -80°C. The residues after removing the appropriate volume for analysis (see below) were secured with parafilm and returned to -80°C for further reference.

DNA and RNA isolation

DNA and RNA isolation was performed using a commercial isolation kit QIAamp DNA Mini Kit (Qiagen, DE) according to the manufacturer's instructions. Vials of individual lots intended for processing were removed from -80°C and quickly thawed in a stream of air at ambient temperature. 500 µl of each sample was pipetted into pre-prepared tubes with Proteinase K and Lysis Buffer. After incubation for 10 minutes at 60°C, the lysate was precipitated with 96°C ethanol and centrifuged through isolation columns. Isolation columns were then washed with buffers with different ethanol contents and the filters were air-dried. DNA and RNA were eluted into 50 µl of Elution Buffer.

4 µl of the DNA/RNA isolate each was used for reverse transcription using the verso cDNA kit (ThermoFisher Scientific, USA) according to the manufacturer's instructions. Reverse transcription was performed at 47°C for 1 hour.

DNA/RNA isolates were stored at -80°C, cDNA at -20°C.

PCR analysis

Multiplex quantitative Real-Time PCR was performed using oligonucleotides specific to:

- mRNA for the S protein (manufacturer-declared molecular target)
- DNA Ori sequence (contained in expression cloning vectors)
- DNA ITS *Escherichia coli* (to detect possible contamination from bacterial DNA used in vector propagation)

Molecular targets for quantitative multiplex Real-Time PCR

The following manufacturer-declared and manufacturer-non-declared molecular targets were used to analyze the content of individual lots at the nucleic acid level:

- mRNA for S protein, manufacturer-declared molecular target; sequence in Spikevax GenBank: OK120841; sequence in BNT162b2 GenBank: OR134577
- DNA Ori contained in the expression cloning vector, GenBank: OR134577
- DNA ITS *Escherichia coli* – Internal Transcribed Spacer in the 16S rDNA cassette, GenBank: AP027563, to assess the level of possible contamination of the samples with genomic DNA of *Escherichia coli* used for the propagation of expression vectors during the manufacturing processes.

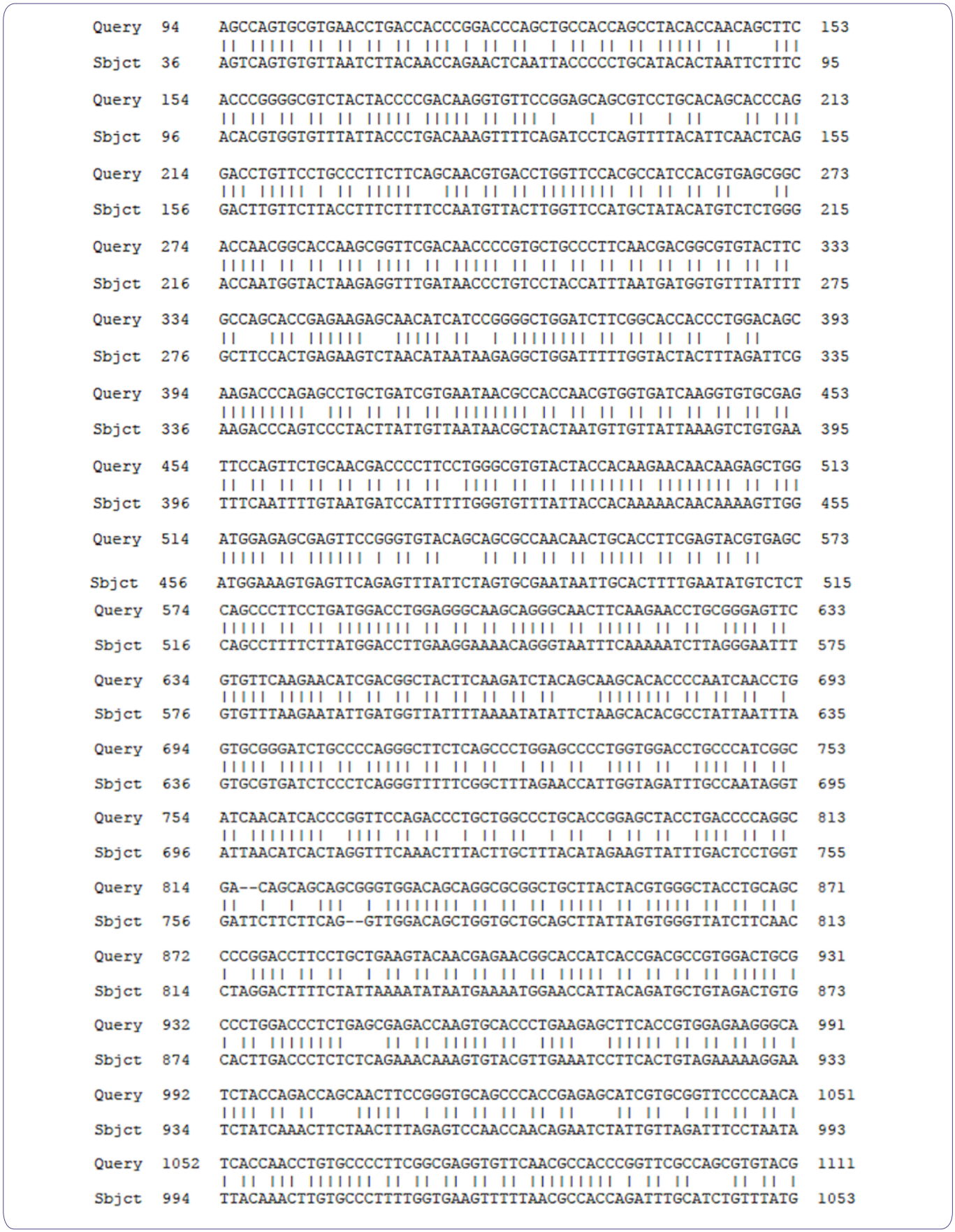
Genomic reference sequences were obtained from GenBank, including

- Original Wuhan SARS-CoV-2 reference strain sequence (GenBank: MT192773) [6]
- Spikevax mRNA sequence (GenBank: OK120841) [7]
- BNT162b2 mRNA sequence (GenBank: OR134577) [8]

Results

Nucleic acid identification

PCR analysis confirmed the presence of mRNA sequences corresponding to the declared Spikevax and BNT162b2 vaccine profiles. Sequence alignments demonstrated significant differences between the vaccine mRNA sequences and the original Wuhan SARS-CoV-2 spike (S) protein mRNA sequence (GenBank: MT192773). These differences reflect intentional modifications made to enhance mRNA stability and translation efficiency. Despite these engineered differences, the encoded S protein in both vaccines contained only two amino acid substitutions (K986P and V987P) relative to the Wuhan strain. This stabilization strategy is consistent with manufacturer documentation.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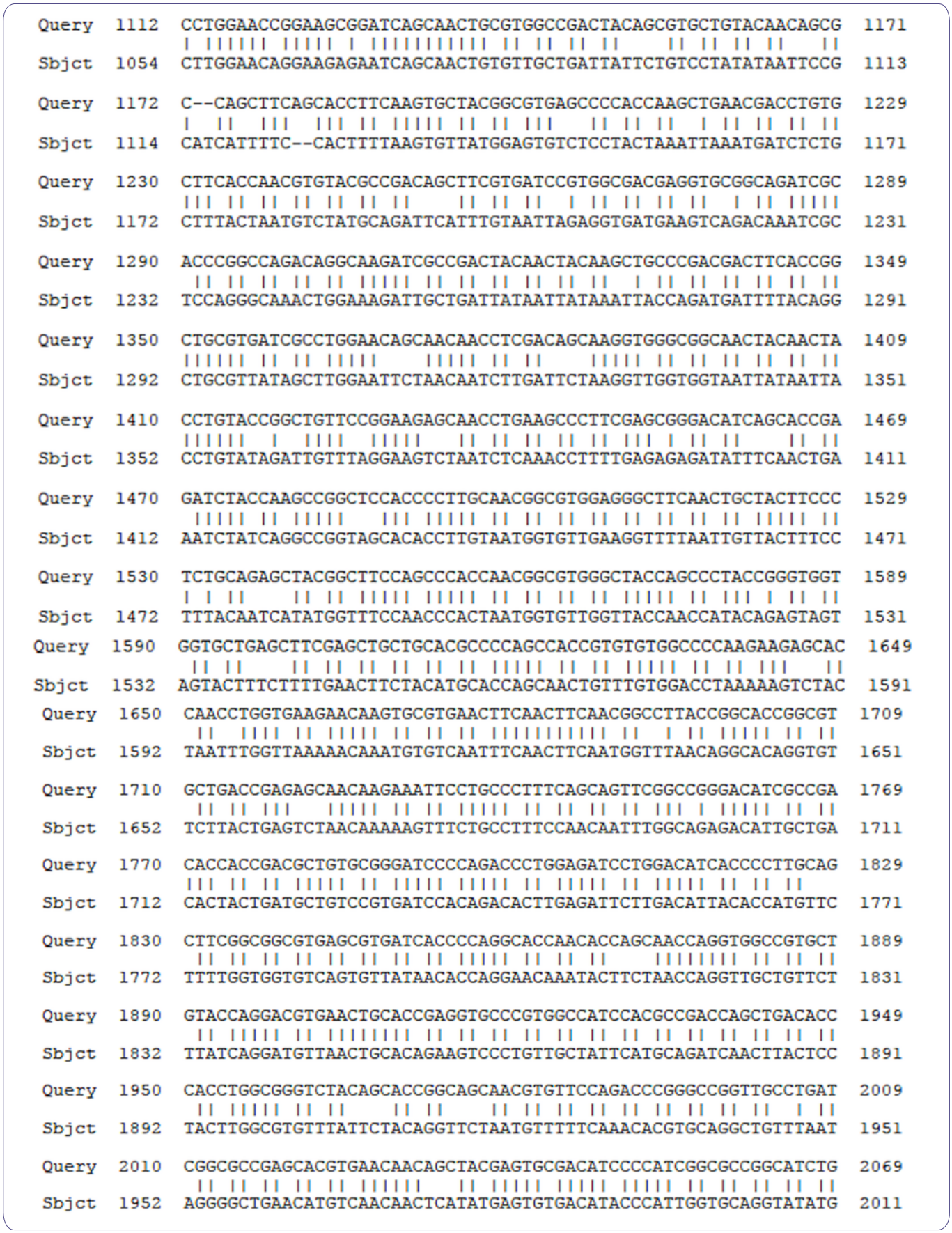
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Sbjct  3812  TTACAC  3817
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Figure 1: Comparison of the mRNA for the S protein in Spikevax (Query) and the mRNA for the S protein in the original Wuhan SARS-CoV-2 (Subject). Vertical lines connect identical bases; the significant difference between the two mRNA sequences is evident.

Query: Figure_2_32321_Spike-encoding_contig_assembled_from_Moderna_mRNA-1273_vaccine Query ID:

lcl|Query_239423 Length: 4004

>MT192773 Wuhan SARS-CoV-2 reference sequence, S protein

Sequence ID: Query_239425 Length: 3821

Range 1: 36 to 3817

Score:1484 bits (1645), Expect:0.0,

Identities:2603/3786(69%), Gaps:8/3786(0%), Strand: Plus/Plus

```
Query  56    TGTTCGTGTTCCTGGTGCTGCTGCCTCTGGTGTCCAGCCAGTGTGTGAACCTGACCACCA  115
             |||| || || || ||  |  |||| || || || || ||||||||| || || || ||||
Sbjct  1     TGTTTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCA  60

Query  116   GAACACAGCTGCCTCCAGCCTACACCAACAGCTTTACCAGAGGCGTGTACTACCCCGACA  175
             |||| ||  | || || || ||||| ||    || ||  | || || || ||||| ||||
Sbjct  61    GAACTCAATTACCCCCTGCATACACTAATTCTTTCACACGTGGTGTTTATTACCCTGACA  120

Query  176   AGGTGTTCAGATCCAGCGTGCTGCACTCTACCCAGGACCTGTTCCTGCCTTTCTTCAGCA  235
             | || |||||||||   ||  | || || || ||||||  |||||  | |||||||||  ||
Sbjct  121   AAGTTTTCAGATCCTCAGTTTTACATTCAACTCAGGACTTGTTCTTACCTTTCTTTTCCA  180

Query  236   ACGTGACCTGGTTCCACGCCATCCACGTGTCCGGCACCAATGGCACCAAGAGATTCGACA  295
             | || || ||||||||| || || || || || || |||||||| || |||||  || || |
Sbjct  181   ATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAATGGTACTAAGAGGTTTGATA  240

Query  296   ACCCCGTGCTGCCCTTCAACGACGGGGTGTACTTTGCCAGCACCGAGAAGTCCAACATCA  355
             |||| || || || || || || || || || || |||||   ||| |||||||| ||||| |
Sbjct  241   ACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCACTGAGAAGTCTAACATAA  300

Query  356   TCAGAGGCTGGATCTTCGGCACCACACTGGACAGCAAGACCCAGAGCCTGCTGATCGTGA  415
             | |||||||||| || || || ||  | ||    |||||||||  ||| || || || |
Sbjct  301   TAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACTTATTGTTA  360

Query  416   ACAACGCCACCAACGTGGTCATCAAAGTGTGCGAGTTCCAGTTCTGCAACGACCCCTTCC  475
             | ||||| || || || || || ||||| || || || || || || || || || || ||
Sbjct  361   ATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTT  420

Query  476   TGGGCGTCTACTACCACAAGAACAACAAGAGCTGGATGGAAAGCGAGTTCCGGGTGTACA  535
             |||| || || |||||||| |||||||| || ||||||||||| |||||| | || ||
Sbjct  421   TGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATT  480

Query  536   GCAGCGCCAACAACTGCACCTTCGAGTACGTGTCCCAGCCTTTCCTGATGGACCTGGAAG  595
               || || || || ||||| || || || || || |||||||| || |||||||| ||||
Sbjct  481   CTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAG  540

Query  596   GCAAGCAGGGCAACTTCAAGAACCTGCGCGAGTTCGTGTTTAAGAACATCGACGGCTACT  655
             | || ||||| || ||||| || ||  | || || |||||||||| || || || || |
Sbjct  541   GAAAACAGGGTAATTTCAAAAATCTTAGGGAATTTGTGTTTAAGAATATTGATGGTTATT  600

Query  656   TCAAGATCTACAGCAAGCACACCCCTATCAACCTCGTGCGGGATCTGCCTCAGGGCTTCT  715
             | || || ||    |||||||| ||||| ||  | ||||| ||||| |||||||| || |
Sbjct  601   TTAAAATATATTCTAAGCACACGCCTATTAATTTAGTGCGTGATCTCCCTCAGGGTTTTT  660

Query  716   CTGCTCTGGAACCCCTGGTGGATCTGCCCATCGGCATCAACATCACCCGGTTTCAGACAC  775
             | ||| | |||||  |||| ||| |||| || || || ||||||||  ||||||| ||
Sbjct  661   CGGCTTTAGAACCATTGGTAGATTTGCCAATAGGTATTAACATCACTAGGTTTCAAACTT  720

Query  776   TGCTGGCCCTGCACAGAAGCTACCTGACACCTGGCGATAGCAGCAGC-GGATGGACAGCT  834
             | || ||  | || ||||| ||  |||| ||||| |||  |  |  | || |||||||||
Sbjct  721   TACTTGCTTTACATAGAAGTTATTTGACTCCTGGTGATT-CTTCTTCAGGTTGGACAGCT  779

Query  835   GGTGCCGCCGCTTACTATGTGGGCTACCTGCAGCCTAGAACCTTCCTGCTGAAGTACAAC  894
             ||||| || ||||| |||||||| || || || ||||| || || ||  | || || ||
Sbjct  780   GGTGCTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTATTAAAATATAAT  839

Query  895   GAGAACGGCACCATCACCGACGCCGTGGATTGTGCTCTGGATCCTCTGAGCGAGACAAAG  954
             || || || ||||| || || || || || ||||| || || |||||    || ||||||
Sbjct  840   GAAAATGGAACCATTACAGATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAG  899

Query  955   TGCACCCTGAAGTCCTTCACCGTGGAAAAGGGCATCTACCAGACCAGCAACTTCCGGGTG  1014
             || ||  |||| |||||||| || ||||| || ||||| || ||    |||||  | ||
Sbjct  900   TGTACGTTGAAATCCTTCACTGTAGAAAAAGGAATCTATCAAACTTCTAACTTTAGAGTC  959

Query  1015  CAGCCCACCGAATCCATCGTGCGGTTCCCCAATATCACCAATCTGTGCCCCTTCGGCGAG  1074
             || || || ||||| || ||  | || || ||||| || ||  ||||||| || || ||
Sbjct  960   CAACCAACAGAATCTATTGTTAGATTTCCTAATATTACAAACTTGTGCCCTTTTGGTGAA  1019

Query  1075  GTGTTCAATGCCACCAGATTCGCCTCTGTGTACGCCTGGAACCGGAAGCGGATCAGCAAT  1134
             || || || |||||||||||| || ||||| || || |||||| ||||| | ||||||||
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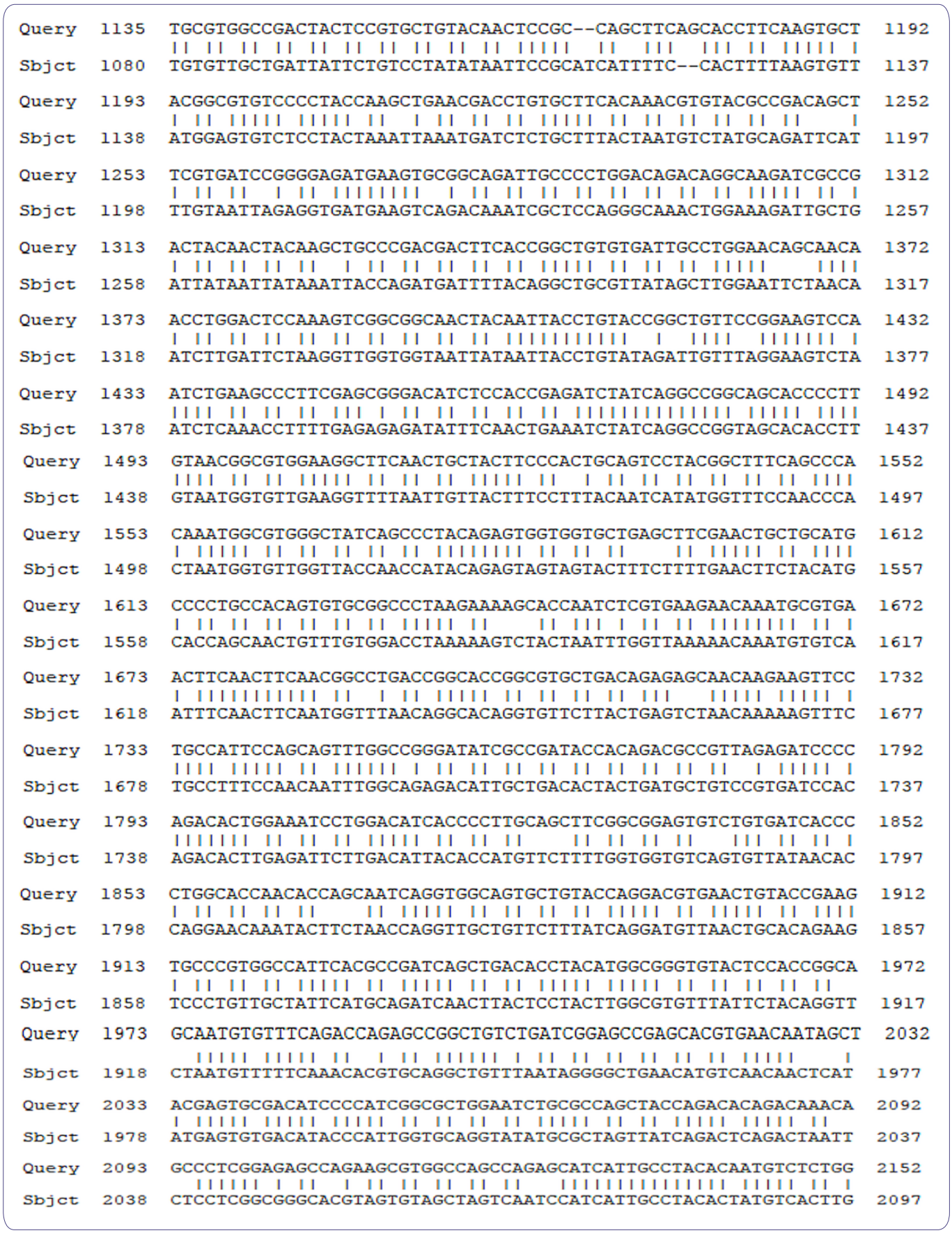
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Query 1193 ACGGCGTGTCCCCTACCAAGCTGAACGACCTGTGCTTCACAAACGTGTACGCCGACAGCT 1252
Sbjct 1138 ATGGAGTGTCTCCTACTAAATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCAT 1197
Query 1253 TCGTGATCCGGGGAGATGAAGTGCGGCAGATTGCCCCTGGACAGACAGGCAAGATCGCCG 1312
Sbjct 1198 TTGTAATTAGAGGTGATGAAGTCAGACAAATCGCTCCAGGGCAAACTGGAAAGATTGCTG 1257
Query 1313 ACTACAACTACAAGCTGCCCGACGACTTCACCGGCTGTGTGATTGCCTGGAACAGCAACA 1372
Sbjct 1258 ATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGTTATAGCTTGGAATTCTAACA 1317
Query 1373 ACCTGGACTCCAAAGTCGGCGGCAACTACAATTACCTGTACCGGCTGTTCCGGAAGTCCA 1432
Sbjct 1318 ATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTA 1377
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Sbjct 1378 ATCTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTT 1437
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Sbjct 1438 GTAATGGTGTTGAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCA 1497
Query 1553 CAAATGGCGTGGGCTATCAGCCCTACAGAGTGGTGGTGCTGAGCTTCGAACTGCTGCATG 1612
Sbjct 1498 CTAATGGTGTTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACTTCTACATG 1557
Query 1613 CCCCTGCCACAGTGTGCGGCCCTAAGAAAAGCACCAATCTCGTGAAGAACAAATGCGTGA 1672
Sbjct 1558 CACCAGCAACTGTTTGTGGACCTAAAAAGTCTACTAATTTGGTTAAAAACAAATGTGTCA 1617
Query 1673 ACTTCAACTTCAACGGCCTGACCGGCACCGGCGTGCTGACAGAGAGCAACAAGAAGTTCC 1732
Sbjct 1618 ATTTCAACTTCAATGGTTTAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTTTC 1677
Query 1733 TGCCATTCCAGCAGTTTGGCCGGGATATCGCCGATACCACAGACGCCGTTAGAGATCCCC 1792
Sbjct 1678 TGCCTTTCCAACAATTTGGCAGAGACATTGCTGACACTACTGATGCTGTCCGTGATCCAC 1737
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Sbjct 1738 AGACACTTGAGATTCTTGACATTACACCATGTTCTTTTGGTGGTGTCAGTGTTATAACAC 1797
Query 1853 CTGGCACCAACACCAGCAATCAGGTGGCAGTGCTGTACCAGGACGTGAACTGTACCGAAG 1912
Sbjct 1798 CAGGAACAAATACTTCTAACCAGGTTGCTGTTCTTTATCAGGATGTTAACTGCACAGAAG 1857
Query 1913 TGCCCGTGGCCATTCACGCCGATCAGCTGACACCTACATGGCGGGTGTACTCCACCGGCA 1972
Sbjct 1858 TCCCTGTTGCTATTCATGCAGATCAACTTACTCCTACTTGGCGTGTTTATTCTACAGGTT 1917
Query 1973 GCAATGTGTTTCAGACCAGAGCCGGCTGTCTGATCGGAGCCGAGCACGTGAACAATAGCT 2032
Sbjct 1918 CTAATGTTTTTCAAACACGTGCAGGCTGTTTAATAGGGGCTGAACATGTCAACAACTCAT 1977
Query 2033 ACGAGTGCGACATCCCCATCGGCGCTGGAATCTGCGCCAGCTACCAGACACAGACAAACA 2092
Sbjct 1978 ATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATT 2037
Query 2093 GCCCTCGGAGAGCCAGAAGCGTGGCCAGCCAGAGCATCATTGCCTACACAATGTCTCTGG 2152
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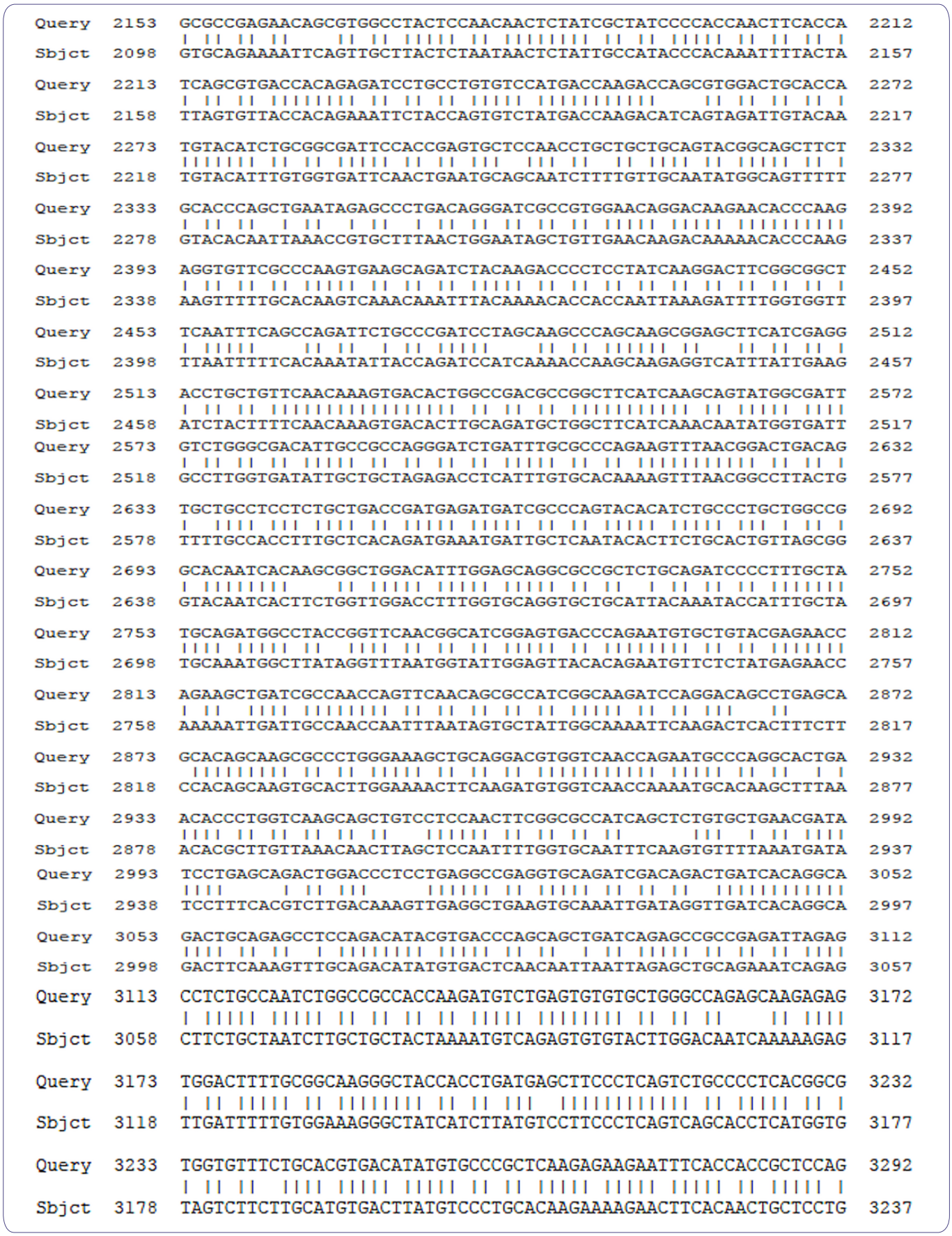
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Sbjct 2098 GTGCAGAAAATTCAGTTGCTTACTCTAATAACTCTATTGCCATACCCACAAATTTTACTA 2157
Query 2213 TCAGCGTGACCACAGAGATCCTGCCTGTGTCCATGACCAAGACCAGCGTGGACTGCACCA 2272
Sbjct 2158 TTAGTGTTACCACAGAAATTCTACCAGTGTCTATGACCAAGACATCAGTAGATTGTACAA 2217
Query 2273 TGTACATCTGCGGCGATTCCACCGAGTGCTCCAACCTGCTGCTGCAGTACGGCAGCTTCT 2332
Sbjct 2218 TGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTTTTGTTGCAATATGGCAGTTTTT 2277
Query 2333 GCACCCAGCTGAATAGAGCCCTGACAGGGATCGCCGTGGAACAGGACAAGAACACCCAAG 2392
Sbjct 2278 GTACACAATTAAACCGTGCTTTAACTGGAATAGCTGTTGAACAAGACAAAAACACCCAAG 2337
Query 2393 AGGTGTTCGCCCAAGTGAAGCAGATCTACAAGACCCCTCCTATCAAGGACTTCGGCGGCT 2452
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Query 2453 TCAATTTCAGCCAGATTCTGCCCGATCCTAGCAAGCCCAGCAAGCGGAGCTTCATCGAGG 2512
Sbjct 2398 TTAATTTTTCACAAATATTACCAGATCCATCAAAACCAAGCAAGAGGTCATTTATTGAAG 2457
Query 2513 ACCTGCTGTTCAACAAAGTGACACTGGCCGACGCCGGCTTCATCAAGCAGTATGGCGATT 2572
Sbjct 2458 ATCTACTTTTCAACAAAGTGACACTTGCAGATGCTGGCTTCATCAAACAATATGGTGATT 2517
Query 2573 GTCTGGGCGACATTGCCGCCAGGGATCTGATTTGCGCCCAGAAGTTTAACGGACTGACAG 2632
Sbjct 2518 GCCTTGGTGATATTGCTGCTAGAGACCTCATTTGTGCACAAAAGTTTAACGGCCTTACTG 2577
Query 2633 TGCTGCCTCCTCTGCTGACCGATGAGATGATCGCCCAGTACACATCTGCCCTGCTGGCCG 2692
Sbjct 2578 TTTTGCCACCTTTGCTCACAGATGAAATGATTGCTCAATACACTTCTGCACTGTTAGCGG 2637
Query 2693 GCACAATCACAAGCGGCTGGACATTTGGAGCAGGCGCCGCTCTGCAGATCCCCTTTGCTA 2752
Sbjct 2638 GTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCTGCATTACAAATACCATTTGCTA 2697
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Sbjct 2698 TGCAAATGGCTTATAGGTTTAATGGTATTGGAGTTACACAGAATGTTCTCTATGAGAACC 2757
Query 2813 AGAAGCTGATCGCCAACCAGTTCAACAGCGCCATCGGCAAGATCCAGGACAGCCTGAGCA 2872
Sbjct 2758 AAAAATTGATTGCCAACCAATTTAATAGTGCTATTGGCAAAATTCAAGACTCACTTTCTT 2817
Query 2873 GCACAGCAAGCGCCCTGGGAAAGCTGCAGGACGTGGTCAACCAGAATGCCCAGGCACTGA 2932
Sbjct 2818 CCACAGCAAGTGCACTTGGAAAACTTCAAGATGTGGTCAACCAAAATGCACAAGCTTTAA 2877
Query 2933 ACACCCTGGTCAAGCAGCTGTCCTCCAACTTCGGCGCCATCAGCTCTGTGCTGAACGATA 2992
Sbjct 2878 ACACGCTTGTTAAACAACTTAGCTCCAATTTTGGTGCAATTTCAAGTGTTTTAAATGATA 2937
Query 2993 TCCTGAGCAGACTGGACCCTCCTGAGGCCGAGGTGCAGATCGACAGACTGATCACAGGCA 3052
Sbjct 2938 TCCTTTCACGTCTTGACAAAGTTGAGGCTGAAGTGCAAATTGATAGGTTGATCACAGGCA 2997
Query 3053 GACTGCAGAGCCTCCAGACATACGTGACCCAGCAGCTGATCAGAGCCGCCGAGATTAGAG 3112
Sbjct 2998 GACTTCAAAGTTTGCAGACATATGTGACTCAACAATTAATTAGAGCTGCAGAAATCAGAG 3057
Query 3113 CCTCTGCCAATCTGGCCGCCACCAAGATGTCTGAGTGTGTGCTGGGCCAGAGCAAGAGAG 3172
Sbjct 3058 CTTCTGCTAATCTTGCTGCTACTAAAATGTCAGAGTGTGTACTTGGACAATCAAAAAGAG 3117
Query 3173 TGGACTTTTGCGGCAAGGGCTACCACCTGATGAGCTTCCCTCAGTCTGCCCCTCACGGCG 3232
Sbjct 3118 TTGATTTTTGTGGAAAGGGCTATCATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTG 3177
Query 3233 TGGTGTTTCTGCACGTGACATATGTGCCCGCTCAAGAGAAGAATTTCACCACCGCTCCAG 3292
Sbjct 3178 TAGTCTTCTTGCATGTGACTTATGTCCCTGCACAAGAAAAGAACTTCACAACTGCTCCTG 3237

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Query  3293  CCATCTGCCACGACGGCAAAGCCCACTTTCCTAGAGAAGGCGTGTTCGTGTCCAACGGCA  3352
             ||||  ||  ||  ||  ||  |||||  |||||||||  |  |||||  ||  ||  ||  ||  ||  ||||
Sbjct  3238  CCATTTGTCATGATGGAAAAGCACACTTTCCTCGTGAAGGTGTCTTTGTTTCAAATGGCA  3297

Query  3353  CCCATTGGTTCGTGACACAGCGGAACTTCTACGAGCCCCAGATCATCACCACCGACAACA  3412
             |  ||  |||||  ||  |||||  ||||  ||  ||  ||  ||  ||  |||||  ||  ||  |||||||
Sbjct  3298  CACACTGGTTTGTAACACAAAGGAATTTTTATGAACCACAAATCATTACTACAGACAACA  3357

Query  3413  CCTTCGTGTCTGGCAACTGCGACGTCGTGATCGGCATTGTGAACAATACCGTGTACGACC  3472
             |  ||  ||||||||  |||||  ||  ||  ||  ||  ||  |||||  |||||  ||  ||  ||  ||  |
Sbjct  3358  CATTTGTGTCTGGTAACTGTGATGTTGTAATAGGAATTGTCAACAACACAGTTTATGATC  3417

Query  3473  CTCTGCAGCCCGAGCTGGACAGCTTCAAAGAGGAACTGGACAAGTACTTTAAGAACCACA  3532
             ||  ||||  ||  ||  |  |||  |||||  |||||  |  ||  ||  ||  ||||||||  ||  |
Sbjct  3418  CTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTAGATAAATATTTTAAGAATCATA  3477

Query  3533  CAAGCCCCGACGTGGACCTGGGCGATATCAGCGGAATCAATGCCAGCGTCGTGAACATCC  3592
             ||    ||  ||  ||  ||  |  ||  ||  |||    ||  ||  |||||     ||  ||  |||||  |
Sbjct  3478  CATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAATGCTTCAGTTGTAAACATTC  3537

Query  3593  AGAAAGAGATCGACCGGCTGAACGAGGTGGCCAAGAATCTGAACGAGAGCCTGATCGACC  3652
             |  |||||  ||  |||||  ||  ||  |||||  |||||||||  |  ||  ||     ||  |||||  |
Sbjct  3538  AAAAAGAAATTGACCGCCTCAATGAGGTTGCCAAGAATTTAAATGAATCTCTCATCGATC  3597

Query  3653  TGCAAGAACTGGGGAAGTACGAGCAGTACATCAAGTGGCCCTGGTACATCTGGCTGGGCT  3712
             |  ||||||||  ||  |||||  ||||||||  ||  ||  |||||  ||||||||  |||||  ||  |
Sbjct  3598  TCCAAGAACTTGGAAAGTATGAGCAGTATATAAAATGGCCATGGTACATTTGGCTAGGTT  3657

Query  3713  TTATCGCCGGACTGATTGCCATCGTGATGGTCACAATCATGCTGTGTTGCATGACCAGCT  3772
             ||||  ||  ||  ||||||||||  ||  |||||  |||||  |||||  ||  ||  ||||||||  |
Sbjct  3658  TTATAGCTGGCTTGATTGCCATAGTAATGGTGACAATTATGCTTTGCTGTATGACCAGTT  3717

Query  3773  GCTGTAGCTGCCTGAAGGGCTGTTGTAGCTGTGGCAGCTGCTGCAAGTTCGACGAGGACG  3832
             |||||||  ||  ||  ||||||||||||   |||||   |||||||||  ||  ||  ||  ||||
Sbjct  3718  GCTGTAGTTGTCTCAAGGGCTGTTGTTCTTGTGGATCCTGCTGCAAATTTGATGAAGACG  3777

Query  3833  ATTCTGAGCCCGTGCTGAAGGGCGTGAAACTGCACTACACAT  3874
             |  ||||||||  |||||  ||  ||  ||  |||  |  ||  |||||||
Sbjct  3778  ACTCTGAGCCAGTGCTCAAAGGAGTCAAATTACATTACACAT  3819
```

Figure 2: Comparison of S protein mRNA in BNT162b2 (Query) and S protein mRNA in the original Wuhan SARS-CoV-2 (Subject). Vertical lines connect identical bases; a significant difference between the two mRNA sequences is evident.

Query: Figure1_032321_Spike-encoding_contig_assembled_from_BioNTech/Pfizer_BNT-162b2_vaccine; Query ID: lcl|Query_510381 Length: 4175; >MT192773 Wuhan SARS-CoV-2 reference sequence, S protein

Sequence ID: Query_510383 Length: 3821, Range 1: 1 to 3819

Score:2115 bits (2345), Expect:0.0,

Identities:2765/3822(72%), Gaps:6/3822(0%), Strand: Plus/Plus

Oligonucleotides for quantitative multiplex Real-Time PCR

The mRNA sequences in Spikevax and BNT162b2 differ not only significantly from each other, but also, from the original SARS-CoV-2 mRNA sequence of the original circulating strain "Wuhan" (GenBank MT192773).

These differences were used to distinguish mRNAs from different sources. mRNAs can have different sequences and will still encode the same protein – the codons (triplets encoding amino acids) can differ for individual amino acids; at least at their third position; codon sequence heterogeneity for amino acids is high.

At the protein level, the S protein in both Spikevax (Moderna) and BNT162b2 (Pfizer) is almost identical and differs from the S protein of the original SARS-CoV-2 "Wuhan" strain that circulated in March 2020 by only two amino acid substitutions, K986P (Lys986Pro) and V987P (Val987Pro). The results are shown in figures 1-5.

```
Query  50    CCGCCACCATGTTCGTGTTCCTGGTGCTGCTGCCCCTGGTGAGCAGCCAGTGCGTGAACC  109
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Query  110   TGACCACCCGGACCCAGCTGCCACCAGCCTACACCAACAGCTTCACCCGGGGCGTCTACT  169
             |||||||| | || |||||||| ||||||||||||||||||||| ||| | ||||| ||||
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Query  170   ACCCCGACAAGGTGTTCCGGAGCAGCGTCCTGCACAGCACCCAGGACCTGTTCCTGCCCT  229
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Sbjct  167   ACCCCGACAAGGTGTTCAGATCCAGCGTGCTGCACTCTACCCAGGACCTGTTCCTGCCTT  226

Query  230   TCTTCAGCAACGTGACCTGGTTCCACGCCATCCACGTGAGCGGCACCAACGGCACCAAGC  289
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Sbjct  227   TCTTCAGCAACGTGACCTGGTTCCACGCCATCCACGTGTCCGGCACCAATGGCACCAAGA  286

Query  290   GGTTCGACAACCCCGTGCTGCCCTTCAACGACGGCGTGTACTTCGCCAGCACCGAGAAGA  349
             | |||||||||||||||||||||||||||||||| |||||||| ||||||||||||||||
Sbjct  287   GATTCGACAACCCCGTGCTGCCCTTCAACGACGGGGTGTACTTTGCCAGCACCGAGAAGT  346

Query  350   GCAACATCATCCGGGGCTGGATCTTCGGCACCACCCTGGACAGCAAGACCCAGAGCCTGC  409
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Sbjct  347   CCAACATCATCAGAGGCTGGATCTTCGGCACCACACTGGACAGCAAGACCCAGAGCCTGC  406
Query  410   TGATCGTGAATAACGCCACCAACGTGGTGATCAAGGTGTGCGAGTTCCAGTTCTGCAACG  469
             |||||||||| |||||||||||||||| ||||| ||||||||||||||||||||||||||
Sbjct  407   TGATCGTGAACAACGCCACCAACGTGGTCATCAAAGTGTGCGAGTTCCAGTTCTGCAACG  466

Query  470   ACCCCTTCCTGGGCGTGTACTACCACAAGAACAACAAGAGCTGGATGGAGAGCGAGTTCC  529
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Sbjct  467   ACCCCTTCCTGGGCGTCTACTACCACAAGAACAACAAGAGCTGGATGGAAAGCGAGTTCC  526

Query  530   GGGTGTACAGCAGCGCCAACAACTGCACCTTCGAGTACGTGAGCCAGCCCTTCCTGATGG  589
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             ||||||| |||||||||||||||||||||||||||| |||||||||| ||||||||||
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Query  650   ACGGCTACTTCAAGATCTACAGCAAGCACACCCCAATCAACCTGGTGCGGGATCTGCCCC  709
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Sbjct  647   ACGGCTACTTCAAGATCTACAGCAAGCACACCCCTATCAACCTCGTGCGGGATCTGCCTC  706

Query  710   AGGGCTTCTCAGCCCTGGAGCCCCTGGTGGACCTGCCCATCGGCATCAACATCACCCGGT  769
             |||||||||| || ||||| |||||||||| ||||||||||||||||||||||||||||
Sbjct  707   AGGGCTTCTCTGCTCTGGAACCCCTGGTGGATCTGCCCATCGGCATCAACATCACCCGGT  766

Query  770   TCCAGACCCTGCTGGCCCTGCACCGGAGCTACCTGACCCCAGGCGACAGCAGCAGCGGGT  829
             | ||||| |||||||||||||| | |||||||||| || ||||| |||||||||| |
Sbjct  767   TTCAGACACTGCTGGCCCTGCACAGAAGCTACCTGACACCTGGCGATAGCAGCAGCGGAT  826
Query  830   GGACAGCAGGCGCGGCTGCTTACTACGTGGGCTACCTGCAGCCCCGGACCTTCCTGCTGA  889
             ||||||| || || || |||||||| |||||||||||||||  | ||||||||||||||
Sbjct  827   GGACAGCTGGTGCCGCCGCTTACTATGTGGGCTACCTGCAGCCTAGAACCTTCCTGCTGA  886

Query  890   AGTACAACGAGAACGGCACCATCACCGACGCCGTGGACTGCGCCCTGGACCCTCTGAGCG  949
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             ||||  ||||||||||||||||  ||||||||||| |||||||||||||||||||||||||
Sbjct  947   AGACAAAGTGCACCCTGAAGTCCTTCACCGTGGAAAAGGGCATCTACCAGACCAGCAACT  1006

Query  1010  TCCGGGTGCAGCCCACCGAGAGCATCGTGCGGTTCCCCAACATCACCAACCTGTGCCCCT  1069
             ||||||||||||||||||||   ||||||||||||||||||| ||||||||  ||||||||||
Sbjct  1007  TCCGGGTGCAGCCCACCGAATCCATCGTGCGGTTCCCCAATATCACCAATCTGTGCCCCT  1066

Query  1070  TCGGCGAGGTGTTCAACGCCACCCGGTTCGCCAGCGTGTACGCCTGGAACCGGAAGCGGA  1129
             |||||||||||||||| ||||||  |  ||||||   |||||||||||||||||||||||||
Sbjct  1067  TCGGCGAGGTGTTCAATGCCACCAGATTCGCCTCTGTGTACGCCTGGAACCGGAAGCGGA  1126

Query  1130  TCAGCAACTGCGTGGCCGACTACAGCGTGCTGTACAACAGCGCCAGCTTCAGCACCTTCA  1189
             |||||||  ||||||||||||||||  ||||||||||||  ||||||||||||||||||||
Sbjct  1127  TCAGCAATTGCGTGGCCGACTACTCCGTGCTGTACAACTCCGCCAGCTTCAGCACCTTCA  1186
Query  1190  AGTGCTACGGCGTGAGCCCCACCAAGCTGAACGACCTGTGCTTCACCAACGTGTACGCCG  1249
             ||||||||||||||  ||| ||||||||||||||||||||||||| ||||||||||||||
Sbjct  1187  AGTGCTACGGCGTGTCCCCTACCAAGCTGAACGACCTGTGCTTCACAAACGTGTACGCCG  1246
Query  1250  ACAGCTTCGTGATCCGTGGCGACGAGGTGCGGCAGATCGCACCCGGCCAGACAGGCAAGA  1309
             ||||||||||||||| || || || ||||||||||| || || || ||||||||||||||
Sbjct  1247  ACAGCTTCGTGATCCGGGGAGATGAAGTGCGGCAGATTGCCCCTGGACAGACAGGCAAGA  1306

Query  1310  TCGCCGACTACAACTACAAGCTGCCCGACGACTTCACCGGCTGCGTGATCGCCTGGAACA  1369
             ||||||||||||||||||||||||||||||||||||||||||||||||| ||||| ||||||||||
Sbjct  1307  TCGCCGACTACAACTACAAGCTGCCCGACGACTTCACCGGCTGTGTGATTGCCTGGAACA  1366

Query  1370  GCAACAACCTCGACAGCAAGGTGGGCGGCAACTACAACTACCTGTACCGGCTGTTCCGGA  1429
             ||||||||||  |||  ||| ||  ||||||||||||||  ||||||||||||||||||||||
Sbjct  1367  GCAACAACCTGGACTCCAAAGTCGGCGGCAACTACAATTACCTGTACCGGCTGTTCCGGA  1426

Query  1430  AGAGCAACCTGAAGCCCTTCGAGCGGGACATCAGCACCGAGATCTACCAAGCCGGCTCCA  1489
             ||  |||  ||||||||||||||||||||||||  ||||||||||| || ||||||  ||
Sbjct  1427  AGTCCAATCTGAAGCCCTTCGAGCGGGACATCTCCACCGAGATCTATCAGGCCGGCAGCA  1486
Query  1490  CCCCTTGCAACGGCGTGGAGGGCTTCAACTGCTACTTCCCTCTGCAGAGCTACGGCTTCC  1549
             |||||||  ||||||||||  ||||||||||||||||||||  ||||||  |||||||||  |
Sbjct  1487  CCCCTTGTAACGGCGTGGAAGGCTTCAACTGCTACTTCCCACTGCAGTCCTACGGCTTTC  1546

Query  1550  AGCCCACCAACGGCGTGGGCTACCAGCCCTACCGGGTGGTGGTGCTGAGCTTCGAGCTGC  1609
             |||||||  ||  ||||||||||  |||||||||  |  ||||||||||||||||||||  ||||
Sbjct  1547  AGCCCACAAATGGCGTGGGCTATCAGCCCTACAGAGTGGTGGTGCTGAGCTTCGAACTGC  1606

Query  1610  TGCACGCCCCAGCCACCGTGTGTGGCCCCAAGAAGAGCACCAACCTGGTGAAGAACAAGT  1669
             ||||  |||||  |||||  |||||  |||||  |||||||  ||  ||||||||||  |
Sbjct  1607  TGCATGCCCCTGCCACAGTGTGCGGCCCTAAGAAAAGCACCAATCTCGTGAAGAACAAAT  1666

Query  1670  GCGTGAACTTCAACTTCAACGGCCTTACCGGCACCGGCGTGCTGACCGAGAGCAACAAGA  1729
             ||||||||||||||||||||||||  ||||||||||||||||||  ||||||||||||
Sbjct  1667  GCGTGAACTTCAACTTCAACGGCCTGACCGGCACCGGCGTGCTGACAGAGAGCAACAAGA  1726
Query  1730  AATTCCTGCCCTTTCAGCAGTTCGGCCGGGACATCGCCGACACCACCGACGCTGTGCGGG  1789
             |  ||||||||  ||  ||||||||  ||||||||  ||||||||  |||||  |||||  ||   | |
Sbjct  1727  AGTTCCTGCCATTCCAGCAGTTTGGCCGGGATATCGCCGATACCACAGACGCCGTTAGAG  1786

Query  1790  ATCCCCAGACCCTGGAGATCCTGGACATCACCCCTTGCAGCTTCGGCGGCGTGAGCGTGA  1849
             ||||||||||  |||||  ||||||||||||||||||||||||||||||||  |||   ||||
Sbjct  1787  ATCCCCAGACACTGGAAATCCTGGACATCACCCCTTGCAGCTTCGGCGGAGTGTCTGTGA  1846
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Query  1850  TCACCCCAGGCACCAACACCAGCAACCAGGTGGCCGTGCTGTACCAGGACGTGAACTGCA  1909
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Sbjct  1847  TCACCCCTGGCACCAACACCAGCAATCAGGTGGCAGTGCTGTACCAGGACGTGAACTGTA  1906

Query  1910  CCGAGGTGCCCGTGGCCATCCACGCCGACCAGCTGACACCCACCTGGCGGGTCTACAGCA  1969
             |||| ||||||||||||||| |||||||| |||||||||| || |||||||| |||  ||
Sbjct  1907  CCGAAGTGCCCGTGGCCATTCACGCCGATCAGCTGACACCTACATGGCGGGTGTACTCCA  1966

Query  1970  CCGGCAGCAACGTGTTCCAGACCCGGGCCGGTTGCCTGATCGGCGCCGAGCACGTGAACA  2029
             |||||||||| ||||| |||||| | ||||| || |||||||| ||||||||||||||||
Sbjct  1967  CCGGCAGCAATGTGTTTCAGACCAGAGCCGGCTGTCTGATCGGAGCCGAGCACGTGAACA  2026

Query  2030  ACAGCTACGAGTGCGACATCCCCATCGGCGCCGGCATCTGTGCCAGCTACCAGACCCAGA  2089
             | |||||||||||||||||||||||||||||| || ||||| ||||||||||||| ||||
Sbjct  2027  ATAGCTACGAGTGCGACATCCCCATCGGCGCTGGAATCTGCGCCAGCTACCAGACACAGA  2086
Query  2090  CCAATTCACCCCGGAGGGCAAGGAGCGTGGCCAGCCAGAGCATCATCGCCTACACCATGA  2149
             | ||    || ||||| || || |||||||||||||||||||||| |||||||| |||
Sbjct  2087  CAAACAGCCCTCGGAGAGCCAGAAGCGTGGCCAGCCAGAGCATCATTGCCTACACAATGT  2146

Query  2150  GCCTGGGCGCCGAGAACAGCGTGGCCTACAGCAACAACAGCATCGCCATCCCCACCAACT  2209
               |||||||||||||||||||||||||||||  |||||||   ||||| ||||||||||||
Sbjct  2147  CTCTGGGCGCCGAGAACAGCGTGGCCTACTCCAACAACTCTATCGCTATCCCCACCAACT  2206

Query  2210  TCACCATCAGCGTGACCACCGAGATTCTGCCCGTGAGCATGACCAAGACCAGCGTGGACT  2269
             |||||||||||||||||| ||||| ||||| ||| |||||||||||||||||||||||||
Sbjct  2207  TCACCATCAGCGTGACCACAGAGATCCTGCCTGTGTCCATGACCAAGACCAGCGTGGACT  2266

Query  2270  GCACCATGTACATCTGCGGCGACAGCACCGAGTGCAGCAACCTGCTGCTGCAGTACGGCA  2329
             |||||||||||||||||||||   |||||||||  ||||||||||||||||||||||||
Sbjct  2267  GCACCATGTACATCTGCGGCGATTCCACCGAGTGCTCCAACCTGCTGCTGCAGTACGGCA  2326

Query  2330  GCTTCTGCACCCAGCTGAACCGGGCCCTGACCGGCATCGCCGTGGAGCAGGACAAGAACA  2389
             ||||||||||||||||||  | |||||||| || |||||||||| ||||||||||||
Sbjct  2327  GCTTCTGCACCCAGCTGAATAGAGCCCTGACAGGGATCGCCGTGGAACAGGACAAGAACA  2386
Query  2390  CCCAGGAGGTGTTCGCCCAGGTGAAGCAGATCTACAAGACCCCTCCCATCAAGGACTTCG  2449
             |||| |||||||||||||| ||||||||||||||||||||||||| ||||||||||||
Sbjct  2387  CCCAAGAGGTGTTCGCCCAAGTGAAGCAGATCTACAAGACCCCTCCTATCAAGGACTTCG  2446

Query  2450  GCGGCTTCAACTTCAGCCAGATCCTGCCCGACCCCAGCAAGCCCAGCAAGCGGAGCTTCA  2509
             |||||||||| |||||||||| |||||||| || ||||||||||||||||||||||||||
Sbjct  2447  GCGGCTTCAATTTCAGCCAGATTCTGCCCGATCCTAGCAAGCCCAGCAAGCGGAGCTTCA  2506

Query  2510  TCGAGGACCTGCTGTTCAACAAGGTGACCCTAGCCGACGCCGGCTTCATCAAGCAGTACG  2569
             |||||||||||||||||||| ||||| || |||||||||||||||||||||||||||| |
Sbjct  2507  TCGAGGACCTGCTGTTCAACAAAGTGACACTGGCCGACGCCGGCTTCATCAAGCAGTATG  2566

Query  2570  GCGACTGCCTCGGCGACATAGCCGCCCGGGACCTGATCTGCGCCCAGAAGTTCAACGGCC  2629
             |||| || || |||||||| |||||| |||| ||||| |||||||||||||| ||||| |
Sbjct  2567  GCGATTGTCTGGGCGACATTGCCGCCAGGGATCTGATTTGCGCCCAGAAGTTTAACGGAC  2626

Query  2630  TGACCGTGCTGCCTCCCCTGCTGACCGACGAGATGATCGCCCAGTACACCAGCGCCCTGT  2689
             |||| |||||||||| |||||||||| |||||||||||||||||||    ||||||
Sbjct  2627  TGACAGTGCTGCCTCCTCTGCTGACCGATGAGATGATCGCCCAGTACACATCTGCCCTGC  2686
Query  2690  TAGCCGGAACCATCACCAGCGGCTGGACTTTCGGCGCTGGAGCCGCTCTGCAGATCCCCT  2749
             | ||||| || ||||| |||||||||| || || || || ||||||||||||||||||||
Sbjct  2687  TGGCCGGCACAATCACAAGCGGCTGGACATTTGGAGCAGGCGCCGCTCTGCAGATCCCCT  2746

Query  2750  TCGCCATGCAGATGGCCTACCGGTTCAACGGCATCGGCGTGACCCAGAACGTGCTGTACG  2809
             | || ||||||||||||||||||||||||||||||| |||||||||| ||||||||||
Sbjct  2747  TTGCTATGCAGATGGCCTACCGGTTCAACGGCATCGGAGTGACCCAGAATGTGCTGTACG  2806
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Query  2810  AGAACCAGAAGCTGATCGCCAACCAGTTCAACAGCGCCATCGGCAAGATCCAGGACAGCC  2869
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2807  AGAACCAGAAGCTGATCGCCAACCAGTTCAACAGCGCCATCGGCAAGATCCAGGACAGCC  2866

Query  2870  TGAGCAGCACCGCTAGCGCCCTGGGCAAGCTGCAGGACGTGGTGAACCAGAACGCCCAGG  2929
             |||||||||| || |||||||||| |||||||||||||||| |||||||| |||||||
Sbjct  2867  TGAGCAGCACAGCAAGCGCCCTGGGAAAGCTGCAGGACGTGGTCAACCAGAATGCCCAGG  2926

Query  2930  CCCTGAACACCCTGGTGAAGCAGCTGAGCAGCAACTTCGGCGCCATCAGCAGCGTGCTGA  2989
             | |||||||||||||| |||||||||  |  ||||||||||||||||||   |||||||
Sbjct  2927  CACTGAACACCCTGGTCAAGCAGCTGTCCTCCAACTTCGGCGCCATCAGCTCTGTGCTGA  2986

Query  2990  ACGACATCCTGAGCCGGCTGGACCCTCCCGAGGCCGAGGTGCAGATCGACCGGCTGATCA  3049
             ||||  |||||||||  | ||||||||||  ||||||||||||||||||||  | |||||||
Sbjct  2987  ACGATATCCTGAGCAGACTGGACCCTCCTGAGGCCGAGGTGCAGATCGACAGACTGATCA  3046

Query  3050  CTGGCCGGCTGCAGAGCCTGCAGACCTACGTGACCCAGCAGCTGATCCGGGCCGCCGAGA  3109
             | ||| | |||||||||||  |||||  ||||||||||||||||||||  | ||||||||||
Sbjct  3047  CAGGCAGACTGCAGAGCCTCCAGACATACGTGACCCAGCAGCTGATCAGAGCCGCCGAGA  3106

Query  3110  TTCGGGCCAGCGCCAACCTGGCCGCCACCAAGATGAGCGAGTGCGTGCTGGGCCAGAGCA  3169
             ||  |  |||    |||||  ||||||||||||||||||    |||||  ||||||||||||||||
Sbjct  3107  TTAGAGCCTCTGCCAATCTGGCCGCCACCAAGATGTCTGAGTGTGTGCTGGGCCAGAGCA  3166

Query  3170  AGCGGGTGGACTTCTGCGGCAAGGGCTACCACCTGATGAGCTTTCCCCAGAGCGCACCCC  3229
             ||  |  ||||||||  ||||||||||||||||||||||||||||||  ||  |||    ||  ||  |
Sbjct  3167  AGAGAGTGGACTTTTGCGGCAAGGGCTACCACCTGATGAGCTTCCCTCAGTCTGCCCCTC  3226

Query  3230  ACGGAGTGGTGTTCCTGCACGTGACCTACGTGCCCGCCCAGGAGAAGAACTTCACCACCG  3289
             ||||  ||||||||  ||||||||||  ||  ||||||||  ||  ||||||||  ||||||||||
Sbjct  3227  ACGGCGTGGTGTTTCTGCACGTGACATATGTGCCCGCTCAAGAGAAGAATTTCACCACCG  3286

Query  3290  CCCCAGCCATCTGCCACGACGGCAAGGCCCACTTTCCCCGGGAGGGCGTGTTCGTGAGCA  3349
             | ||||||||||||||||||||||  ||||||||||  |  ||  ||||||||||||  ||
Sbjct  3287  CTCCAGCCATCTGCCACGACGGCAAAGCCCACTTTCCTAGAGAAGGCGTGTTCGTGTCCA  3346

Query  3350  ACGGCACCCACTGGTTCGTGACCCAGCGGAACTTCTACGAGCCCCAGATCATCACCACCG  3409
             ||||||||||  ||||||||||  ||||||||||||||||||||||||||||||||||||
Sbjct  3347  ACGGCACCCATTGGTTCGTGACACAGCGGAACTTCTACGAGCCCCAGATCATCACCACCG  3406

Query  3410  ACAACACCTTCGTGAGCGGCAACTGCGACGTGGTGATCGGCATCGTGAACAACACCGTGT  3469
             ||||||||||||||   ||||||||||||||  ||||||||||  ||||||||  |||||||
Sbjct  3407  ACAACACCTTCGTGTCTGGCAACTGCGACGTCGTGATCGGCATTGTGAACAATACCGTGT  3466

Query  3470  ACGATCCCCTGCAGCCCGAGCTGGACAGCTTCAAGGAGGAGCTGGACAAGTACTTCAAGA  3529
             ||||  ||  |||||||||||||||||||||||||  |||||  ||||||||||||||  ||||
Sbjct  3467  ACGACCCTCTGCAGCCCGAGCTGGACAGCTTCAAAGAGGAACTGGACAAGTACTTTAAGA  3526

Query  3530  ATCACACCAGCCCCGACGTGGACCTGGGCGACATCAGCGGCATCAACGCCAGCGTGGTGA  3589
             | |||||  |||||||||||||||||||||||  ||||||||  |||||  ||||||||  ||||
Sbjct  3527  ACCACACAAGCCCCGACGTGGACCTGGGCGATATCAGCGGAATCAATGCCAGCGTCGTGA  3586

Query  3590  ACATCCAGAAGGAGATCGATCGGCTGAACGAGGTGGCCAAGAACCTGAACGAGAGCCTGA  3649
             ||||||||||  ||||||||  ||||||||||||||||||||||  ||||||||||||||||
Sbjct  3587  ACATCCAGAAAGAGATCGACCGGCTGAACGAGGTGGCCAAGAATCTGAACGAGAGCCTGA  3646

Query  3650  TCGACCTGCAGGAGCTGGGCAAGTACGAGCAGTACATCAAGTGGCCCTGGTACATCTGGC  3709
             ||||||||||  ||  |||||  ||||||||||||||||||||||||||||||||||||||||
Sbjct  3647  TCGACCTGCAAGAACTGGGGAAGTACGAGCAGTACATCAAGTGGCCCTGGTACATCTGGC  3706

Query  3710  TGGGCTTCATCGCCGGCCTGATCGCCATCGTGATGGTGACCATCAtgctgtgctgcatga  3769
             |||||||  ||||||||  |||||  ||||||||||||||  ||  ||||||||||  |||||||
Sbjct  3707  TGGGCTTTATCGCCGGACTGATTGCCATCGTGATGGTCACAATCATGCTGTGTTGCATGA  3766
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Query  3770  ccagctgctgcagctgcctgaagggctgttgcagctgcggcagctgctgcaAGTTCGACG  3829
             |||||||||| |||||||||||||||||||| ||||| ||||||||||||||||||||||
Sbjct  3767  CCAGCTGCTGTAGCTGCCTGAAGGGCTGTTGTAGCTGTGGCAGCTGCTGCAAGTTCGACG  3826

Query  3830  AGGACGACAGCGAGCCCGTGCTGAAGGGCGTGAAGCTGCACTACACCTGAT  3880
             |||||||    |||||||||||||||||||||| |||||||||| ||||
Sbjct  3827  AGGACGATTCTGAGCCCGTGCTGAAGGGCGTGAAACTGCACTACACATGAT  3877
```

Figure 3: Comparison of mRNA for S protein in Spikevax (Query) and mRNA for S protein in BNT162b2 (Subject). Vertical lines connect identical bases; a significant difference between the two mRNA sequences is evident.

Query: Figure_2_32321_Spike-encoding_contig_assembled_from_Moderna_mRNA-1273_vaccine Query ID: lcl|Query_246871 Length: 4004

>Figure1_032321_Spike-encoding_contig_assembled_from_BioNTech/Pfizer_BNT-162b2_vaccine

Sequence ID: Query_246873 Length: 4175, Range 1: 47 to 3877

Score:5273 bits (5847), Expect:0.0,

Identities:3468/3831(91%), Gaps:0/3831(0%), Strand: Plus/Plus

```
Query  1     MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS  60
             MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
Sbjct  1     MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS  60

Query  61    NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV  120
             NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
Sbjct  61    NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV  120

Query  121   NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE  180
             NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
Sbjct  121   NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE  180

Query  181   GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT  240
             GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
Sbjct  181   GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT  240

Query  241   LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK  300
             LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
Sbjct  241   LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK  300

Query  301   CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN  360
             CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
Sbjct  301   CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN  360

Query  361   CVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIAD  420
             CVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIAD
Sbjct  361   CVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIAD  420

Query  421   YNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPC  480
             YNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPC
Sbjct  421   YNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPC  480

Query  481   NGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVN  540
             NGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVN
Sbjct  481   NGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVN  540

Query  541   FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP  600
             FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP
Sbjct  541   FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP  600
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Query  601   GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSY  660
             GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSY
Sbjct  601   GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSY  660

Query  661   ECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI  720
             ECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI
Sbjct  661   ECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI  720

Query  721   SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE  780
             SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE
Sbjct  721   SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE  780

Query  781   VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC  840
             VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC
Sbjct  781   VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC  840

Query  841   LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM  900
             LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM
Sbjct  841   LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM  900
Query  901   QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN  960
             QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN
Sbjct  901   QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN  960

Query  961   TLVKQLSSNFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA  1020
             TLVKQLSSNFGAISSVLNDILSRLD  EAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA
Sbjct  961   TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA  1020

Query  1021  SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPA  1080
             SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPA
Sbjct  1021  SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPA  1080

Query  1081  ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDP  1140
             ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDP
Sbjct  1081  ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDP  1140

Query  1141  LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL  1200
             LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
Sbjct  1141  LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL  1200

Query  1201  QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD  1260
             QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD
Sbjct  1201  QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD  1260

Query  1261  SEPVLKGVKLHYT  1273
             SEPVLKGVKLHYT
Sbjct  1261  SEPVLKGVKLHYT  1273
```

Figure 4: Comparison of the translated S protein in Spikevax (Query) and the translated S protein in the original Wuhan SARS-CoV-2 (Subject). With the exception of two amino acid substitutions in Spikevax (K986P; Lys986Pro and V987P; Val987Pro), which are highlighted in red, the two sequences are identical at the protein level.

Query: Moderna S protein ID: lcl|Query_22211 Length: 1273

>Sbjct: Wuhan S protein

Sequence ID: Query_22213 Length: 1273

Range 1: 1 to 1273

Score:2630 bits (6818), Expect:0.0,

Method: Compositional matrix adjust.

Identities:1271/1273(99%), Positives:1271/1273(99%), Gaps:0/1273(0%)

```
Query  1     MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQ  66
            MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQ
Sbjct  1     MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQ  66

Query  67    DLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDS  126
            DLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDS
Sbjct  67    DLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDS  126

Query  127   KTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVS  186
            KTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVS
Sbjct  127   KTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVS  186

Query  187   QPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIG  246
            QPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIG
Sbjct  187   QPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIG  246

Query  247   INITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCA  306
            INITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCA
Sbjct  247   INITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCA  306

Query  307   LDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYA  366
            LDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYA
Sbjct  307   LDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYA  366

Query  367   WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAP  426
            WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAP
Sbjct  367   WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAP  426

Query  427   GQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEI  486
            GQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEI
Sbjct  427   GQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEI  486

Query  487   YQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTN  546
            YQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTN
Sbjct  487   YQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTN  546

Query  547   LVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSF  606
            LVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSF
Sbjct  547   LVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSF  606

Query  607   GGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIG  666
            GGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIG
Sbjct  607   GGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIG  666

Query  667   AEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSI  726
            AEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSI
Sbjct  667   AEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSI  726

Query  727   AIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAV  786
            AIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAV
Sbjct  727   AIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAV  786

Query  787   EQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAG  846
            EQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAG
Sbjct  787   EQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAG  846

Query  847   FIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGA  906
            FIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGA
Sbjct  847   FIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGA  906
```

```
Query  907   ALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVV  966
             ALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVV
Sbjct  907   ALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVV  966

Query  967   NQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQL  1026
             NQNAQALNTLVKQLSSNFGAISSVLNDILSRLD  EAEVQIDRLITGRLQSLQTYVTQQL
Sbjct  967   NQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQL  1026

Query  1027  IRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQE  1086
             IRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQE
Sbjct  1027  IRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQE  1086

Query  1087  KNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGI  1146
             KNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGI
Sbjct  1087  KNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGI  1146

Query  1147  VNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKN  1206
             VNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKN
Sbjct  1147  VNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKN  1206

Query  1207  LNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGS  1266
             LNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGS
Sbjct  1207  LNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGS  1266

Query  1267  CCKFDEDDSEPVLKGVKLHYT  1287
             CCKFDEDDSEPVLKGVKLHYT
Sbjct  1267  CCKFDEDDSEPVLKGVKLHYT  1287
```

Figure 5: Comparison of the translated S protein in BNT162b2 (Query) and the translated S protein in the original Wuhan SARS-CoV-2 (Subject). With the exception of two amino acid substitutions in BNT162b2 (K986P; Lys986Pro and V987P; Val987Pro), which are highlighted in red, the two sequences are identical at the protein level.

Query: Pfizer BNT162b2 S protein

>Sbjct: Wuhan S protein

Sequence ID: Query_6285 Length: 1287

Score: 2643 bits (6851), Expect:0.0,

Method: Compositional matrix adjust.,

Identities: 1279/1281 (99%), Positives: 1279/1281 (99%), Gaps: 0/1281 (0 %)

Based upon the bioinformatic analysis a region was identified in the mRNA sequence of Spikevax and BNT162b2 that was sufficiently homologous to allow primers to be designed for amplification of both Spikevax and BNT162b2 S protein mRNAs, and at the same time sufficiently heterologous to allow fluorescently labeled hybridization probes to accurately discriminate between Spikevax and BNT162b2 S protein mRNAs by quantitative Real-Time PCR. The region that was selected is shown in figure 6.

Primers and fluorescently labelled probe for quantitative Real-Time PCR detection of *Escherichia coli* genomic DNA (HEX) were designed to the internal transcribed spacer region (ITS) of the 16S rDNA cassette. The assay has been validated according to ISO 13485 and is routinely diagnostically used by the laboratory.

All primers and fluorescently labeled hybridization probes used were custom synthesized by Eurofins Genomics, DE.

The sequences of the primers and fluorescently labeled hybridization probes used in the multiplex quantitative Real-Time PCR of mRNA and expression cassettes for S protein Spikevax, BNT162b2, expression cloning vector and *Escherichia coli* genomic DNA are shown in table 1.

Primers and the fluorescently labeled probe (Cy5) for quantitative Real-Time PCR of the expression cloning DNA vector map to the Ori promoter region of Pfizer bivalent expression vector BNT162b2 (GenBank OR134577) and their sequences are published elsewhere.

Design and validation of quantitative multiplex Real-Time PCR

Since the declared mRNA in both Spikevax and BNT162b2 is modified with pseudouridine and it is not known to what extent this modification was performed by the manufacturer, it was not possible to have it artificially synthesized, as is a common practice for construction of calibration curves based on precise knowledge of the

sequence, sequence length, and mass of the synthesized target sequence.

Therefore, we used an alternative method where the Spikevax cDNA and BNT162b2 cDNA were diluted in serial log dilutions to assess whether the difference in the amplification curves for a given assay corresponds to a difference of approximately 3 cycles (Ct), which is a parameter of a correctly designed test and optimal reaction efficiency of all reaction components. If the measured difference in Ct values in individual logarithmic dilutions is approximately 3 cycles, i) the measurement results can be considered valid and ii) it is possible to use a universal calibration curve, which we routinely employ for similar purposes in cases when quantitation of an unknown target is needed.

Figure 6: Region near the 3' end of the S protein mRNA of Spikevax (Query) and BNT162b2 (Subject) sequences targeted by quantitative Real-Time PCR. Common forward primer (red), common reverse primer (blue), probe-discriminating region (BNT162b2 in green - FAM, Spikevax in orange - ROX).

The universal calibration curve was created by averaging calibration curves constructed using serial dilutions of synthetic, precisely defined genomic fragments of 50 microorganisms, including ssRNA viruses, dsRNA viruses, ssDNA viruses, dsDNA viruses, bacteria and fungi (molecular targets that the laboratory routinely diagnostically examines using quantitative Real-Time PCR).

Oligonucleotide	Sequence in 5'-3'
Pfi_Mo common F	TGCGGCAAGGGCTACCACCTGATGAG
Pfi_Mo common R	GGTGTTGTCGGTGGTGATGATCTGG
VV_Ori_F	CTACATACCTCGCTCTGCTAATC
VV_Ori_R	GCGCCTTATCCGGTAACTATC
EcoliITS-F03	CACTCAGGCCTACCAAATTTGCA
EcoliITS-R02	TCGCAGTGAACCTTTGCAGGTAC
EcoliITSProbe_15	HEX - CGCATAGCTCCACCATCTCTGTAGTG - BHQ1
Spikevax Probe	ROX - TCCCCAGAGCGCACCCCACGGAGTGGTGTT - BHQ2
VV_Ori_Probe	CY5 - TGCTGCCAGTGGCGATAAGTCGTGTCTT - BHQ2
BNT162b2 Probe	FAM - CCCTCAGTCTGCCCCTCACGGCGTGGTGTT - BHQ1

Table 1: Primes and fluorescent labelling.

The universal calibration curve equation we used in this investigation was

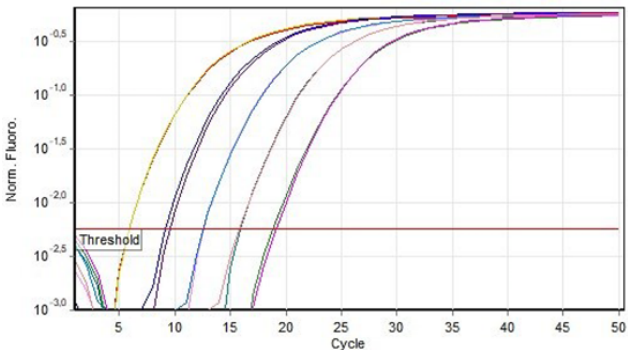
[Eq. 1] conc =10^(-0.257*Ct+11.897).

Using this equation, we calculated the number of copies of the target sequence in a given volume using the obtained Ct values for each individual amplicon.

Quantitative Real-Time PCR was performed using FastStart™ Taq DNA Polymerase, Roche, USA, with 2 mM MgCl2 final. Nucleotides were purchased from Sigma, DE.

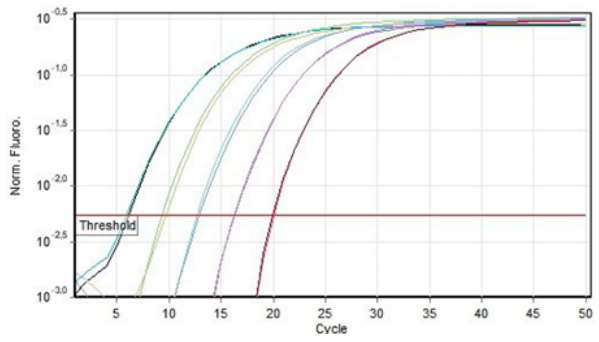
The temperature profile for amplification of all molecular targets in quantitative multiplex Real Time PCR was as follows: 94°C 5 min initial denaturation, then 50 cycles: 94°C for 20 sec, 57°C for 30 sec, then 72°C for 30 sec.

These results for Moderna and Pfizer are shown in figures 7 & 8 respectively.



No.	Color	Sample	Ct
1		Spikevax 200100A - conc cDNA	5.90
2		Spikevax 200100A - conc cDNA	5.91
3		Spikevax 200100A - 10x dil	9.12
4		Spikevax 200100A - 10x dil	9.50
5		Spikevax 200100A - 100x dil	12.59
6		Spikevax 200100A - 100x dil	12.53
7		Spikevax 200100A - 1000x dil	15.88
8		Spikevax 200100A - 1000x dil	15.79
9		Spikevax 200100A - 10000x dil	18.78
10		Spikevax 200100A - 10000x dil	19.09

Figure 7: Spikevax cDNA dilution experiment – concentrated cDNA and 4 serial log dilutions.



No.	Color	Sample	Ct
11		BNT162b2 1F11055A - conc cDNA	6.17
12		BNT162b2 1F11055A - conc cDNA	5.99
13		BNT162b2 1F11055A - 10x dil	9.69
14		BNT162b2 1F11055A - 10x dil	9.32
15		BNT162b2 1F11055A - 100x dil	12.70
16		BNT162b2 1F11055A - 100x dil	12.92
17		BNT162b2 1F11055A - 1000x dil	16.28
18		BNT162b2 1F11055A - 1000x dil	16.24
19		BNT162b2 1F11055A - 10000x dil	20.02
20		BNT162b2 1F11055A - 10000x dil	19.91

Figure 8: BNT162b2 cDNA dilution experiment - concentrated cDNA and 4 serial log dilutions.

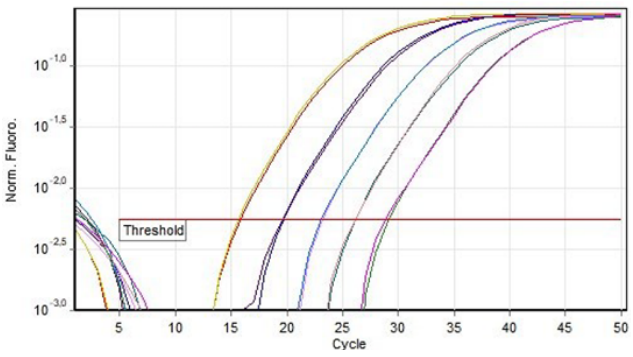
Presence of undeclared DNA sequences

A notable observation made during this initial validation was that the cDNA isolates were heavily contaminated with the expression cloning vector.

These unexpected DNA sequences were identified in several vaccine lots. Analysis confirmed the presence of bacterial genomic DNA fragments from Escherichia coli, specifically sequences from the 16S rDNA internal transcribed spacer (ITS) region. These DNA elements are associated with bacterial vectors used during mRNA manufacturing.

For the sake of completeness, we carried out serial log dilution curves of the DNA of the contaminating expression vector, which were measured using the quantitative multiplex Real-Time PCR test used, essentially as an incidental finding against the background of the primary goal - to validate the reaction efficiency of the detection of the manufacturer's declared target, i.e. mRNA for the S protein.

These DNA amplicons also showed acceptable reaction efficiency, based on the measured Ct values in the dilution experiment as shown in figures 9 & 10.



		Sample	Ct
1		Spikevax 200100A - conc cDNA	15.83
2		Spikevax 200100A - conc cDNA	15.65
3		Spikevax 200100A - 10x dil	19.63
4		Spikevax 200100A - 10x dil	19.58
5		Spikevax 200100A - 100x dil	23.21
6		Spikevax 200100A - 100x dil	23.10
7		Spikevax 200100A - 1000x dil	26.12
8		Spikevax 200100A - 1000x dil	26.08
9		Spikevax 200100A - 10000x dil	29.21
10		Spikevax 200100A - 10000x dil	28.86

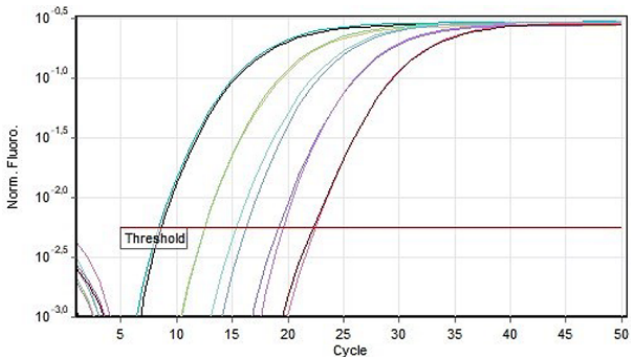
Figure 9: Spikevax cDNA dilution experiment – concentrated cDNA and 4 serial log dilutions. Accidental co-amplification of contaminating genomic DNA of the expression vector.

Oligonucleotides for quantitative Real-Time PCR of the Ori promoter expression cloning Sequencing of bivalent Moderna and Pfizer mRNA vaccines demonstrated nanogram to microgram quantities of expression vector dsDNA per dose. No evidence of SV40 was identified.

Oligonucleotides for quantitative Real-Time PCR of Escherichia coli genomic DNA were validated based on the requirements of ISO 13 485 as a constituent of a commercial diagnostic kit.

The oligonucleotides for quantitative Real-Time PCR cassettes for S protein (mRNA) in Spikevax and BNT162b2 preparations, newly used in this work, were verified by direct sequencing of obtained PCR products on an ABI 3500 capillary sequencer (ThermoFisher Scientific, USA).

All 17 lots of Spikevax and all 7 lots of BNT162b2 were sequenced. Direct sequencing of PCR products was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, USA). Sequencing reactions were purified using the BigDye XTerminator™ Purification Kit (ThermoFisher Scientific, USA), according to the manufacturer's recommended procedure.



		Sample	Ct
11		BNT162b2 1F11055A - conc cDNA	8.57
12		BNT162b2 1F11055A - conc cDNA	8.34
13		BNT162b2 1F11055A - 10x dil	12.50
14		BNT162b2 1F11055A - 10x dil	12.51
15		BNT162b2 1F11055A - 100x dil	15.34
16		BNT162b2 1F11055A - 100x dil	16.22
17		BNT162b2 1F11055A - 1000x dil	19.10
18		BNT162b2 1F11055A - 1000x dil	19.53
19		BNT162b2 1F11055A - 10000x dil	22.49
20		Pfizer 1F11055A - 10000x dil	22.30

Figure 10: BNT162b2 cDNA dilution experiment – concentrated cDNA and 4 serial log dilutions. Accidental co-amplification of contaminating genomic DNA of the expression vector.

One typical example of a fragment of the chromatogram of the Spikevax S protein gene and one typical example of a fragment of the chromatogram of the BNT162b2 S protein gene are shown in figure 11. ABI 3500 sequencing data of all other lots are available upon request.

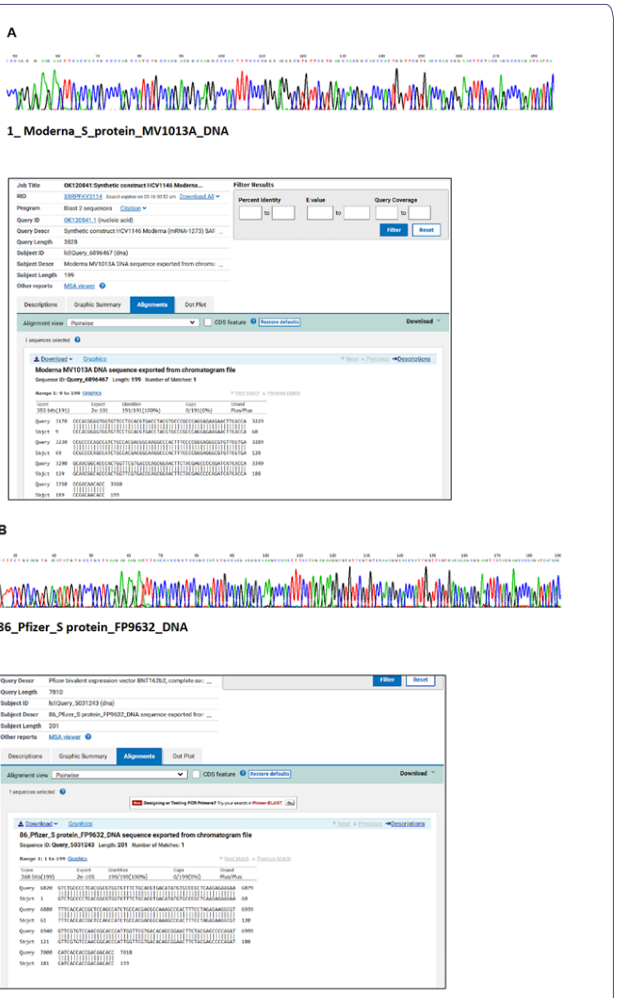


Figure 11: Sanger sequencing chromatograms of obtained PCR products. In all cases, the sequenced PCR products showed 100% identity to the expected reference sequence. A 1_Moderna_S_protein_MV1013A_DNA; B – 86_Pfizer_S protein_FP9632_DNA.

Quantitative real-time PCR analysis of mRNA for S protein

At the mRNA (cDNA) level, it was found that the expression of the declared molecular target (S protein mRNA) varies across lots for both Spikevax and BNT162b2.

For Spikevax (Moderna), as shown in figure 12, lots 200106A and MV1018A are characterized by one order of magnitude (10x) lower expression of S protein mRNA than the other tested lots. The blue dots in the graph indicate the S protein mRNA expression values for individual measurements (in the range of 10e9 to 10e10 copies of the target sequence/ml; analysis performed in quintuple for each lot). The orange dots in this case indicate the quantity of expression cloning vector DNA (in the range of 10e7 to 10e8 copies of the target sequence/ml; analysis performed in quintuple for each lot) present in the analyzed cDNAs. Similar findings are shown in figure 13, lots 1L084A and 1F1059A for Pfizer BNT162b2.

Quantitative Real-Time PCR analysis of DNA targets - S protein cassette and expression cloning vector promoter

A significant finding is the high quantity of DNA present across all tested lots, both Spikevax (Moderna) and BNT162b2 (Pfizer). In all preparations, a very high Real Time PCR signal from the promoter of the cloning expression vector and from the 3' end of the cassette encoding the S protein was measured (10e7 – 10e9 copies/ml for Spikevax; 10e8 – 10e9 copies/ml for BNT162b2). Analysis of all lots was carried out in quintuples with the final results displayed in figures 14 & 15 respectively.

These data suggest that both assays might detect identical DNA construct, targeted at both its 5' end (promoter Ori) and its 3' end (bases 3184 to 3417 of the total 3880 bp of the complete coding sequence for the S protein). This most likely suggests that both Spikevax and BNT162b2 might contain the complete coding DNA sequence for the S protein cassette, together with some regulatory promoter sequences.

The results demonstrate more than degraded or fragmented DNA, that might arise from instability of the stored preparations or during suboptimal manufacturing process. The combined signal originates very likely from a full-length DNA construct, which might be capable of encoding a full-length mRNA for the S protein.

Within the tested lots of Spikevax (Moderna), the heterogeneity in the quantity of DNA coding for the S protein cassette and/or the

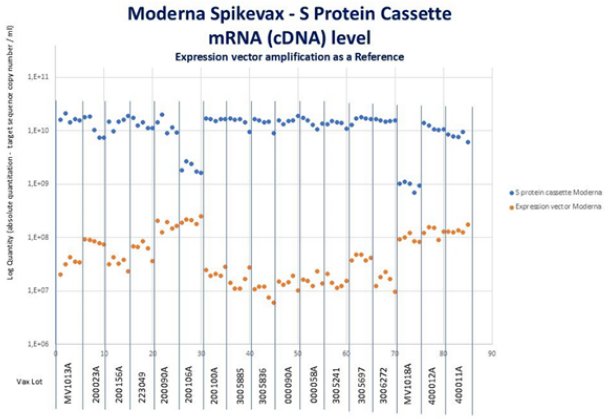


Figure 12: The graph shows the mRNA expression level for S protein in Spikevax (Moderna), GenBank OK120841 - blue dots in quintuplicates for each tested lot. Orange dots serve as a reference; it is an admixture of double-stranded DNA of the expression cloning vector in the tested cDNAs.

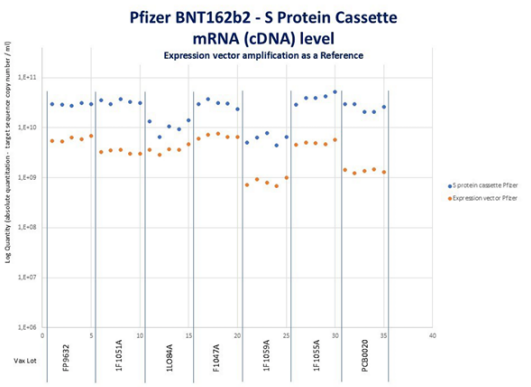


Figure 13: In BNT162b2 (Pfizer), the variation in mRNA expression for the declared S protein (GenBank OR134577) between individual lots is also noticeable – a difference of one order of magnitude (10x) is evident for lots 1L084A and 1F1059A – mRNA expression for S protein is shown in the graph by blue dots. The orange dots serve as a reference; it is an admixture of double-stranded DNA of the expression cloning vector in the tested cDNAs. Analysis of all lots was carried out in quintuples.

cloning expression vector is remarkable. In three cases, the ratio of the amount of expression cloning vector and DNA cassette for the S protein is reversed, and the difference in quantity is of one order of magnitude. This indicates that the given lot might also contain another construct cloned in the given expression vector. It is possible it could represent an admixture of Omicron, although this could mean that its amount is very random across the individual lots, which would raise concerns regarding good manufacturing practice.

We did not search for the Omicron sequence in this investigation. Rather, we focused on sequences that are clearly declared to be in the official content of the preparations – thus, we targeted the S protein mRNA [6], GenBank OK120841 (Spikevax, Moderna) [7]; GenBank OR134577 (BNT162b2, Pfizer) [8].

These differences in the quantity of DNA amplicons are clearly visible already in the raw data from the analyzer (RotorGene Q, Qiagen, Germany). Note the significantly different starts of the amplification curves.

Figure 16 shows the raw data analyzed at the DNA level for the S protein cassette Spikevax and BNT162b2, as well as for the expression cloning vector, which, like the DNA cassette for the S protein, is present in all tested lots.

The summarized results of the mRNA and DNA quantities for the S protein cassette are shown in table 2.

Quantitative detection of genomic DNA of escherichia coli

The presence of *Escherichia coli* genomic DNA was also analyzed within the multiplex quantitative Real-Time PCR to verify whether the preparations were contaminated with GMO, typically used for the propagation of the cloning expression vector harboring the S protein cassette.

A borderline quantity, in individual copies of the microorganism/ml of sample, was found in two lots of BNT162b2 (Pfizer), namely FP9632 and 1F1047A.

The data is not shown, though they can be provided upon request.

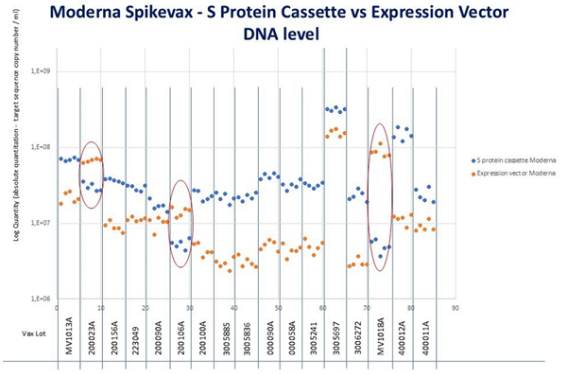


Figure 14: Shows a high quantity of DNA for both the expression cloning vector and the DNA for the S protein cassette. Of note are lots 200023A, 200106A, MV1018A, where the ratios of vector vs cassette are reversed. It is also interesting that lots 3005697 and 400012A are quantitatively significantly outside the range of the other tested lots.

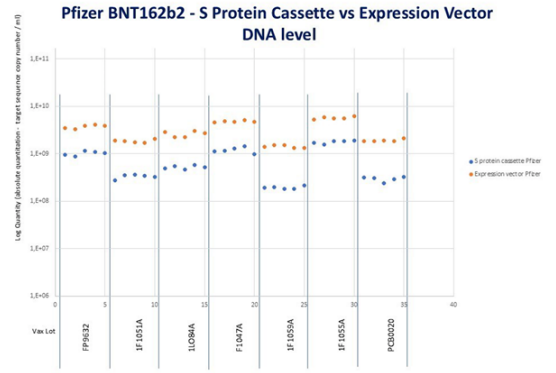


Figure 15: Shows a similar situation for BNT162b2 (Pfizer) preparations. In these preparations, the amount of expression cloning vector is higher, but what is more important is the horizontal heterogeneity within the blue dots, which represent quantitative S protein DNA cassette amplicons. Note the one order of magnitude (10x) difference between the lots.

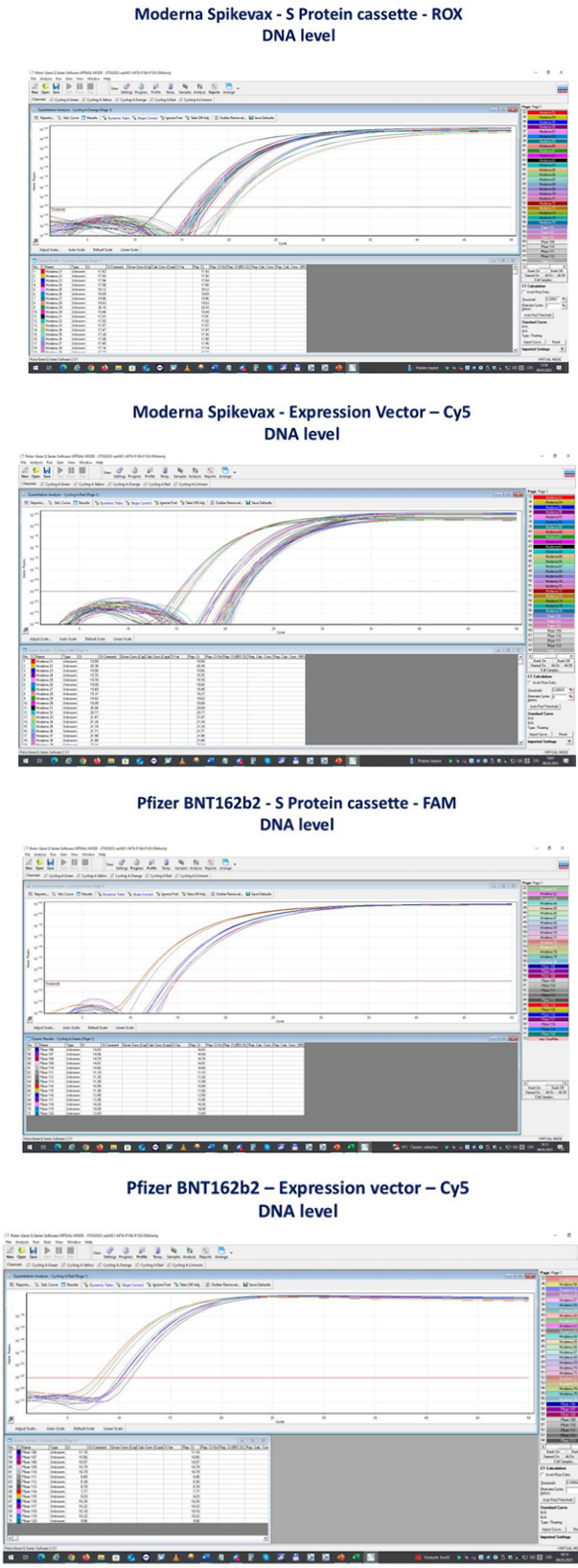


Figure 16: Te following graphic print screens show raw data for the DNA cassette for the S protein (Spikevax - ROX, BNT162b2 – FAM, expression cloning vector – Cy5).

Homogeneity assessment

While there was a 28% batch-to-batch variability in nucleic acid content, there was no significant intra-lot inconsistencies observed. Each set of vials within a given lot showed consistent mRNA and DNA profiles.

Impact of expired storage conditions

Analysis of expired vaccine samples stored at -80°C indicated partial degradation of nucleic acid content. While mRNA fragments remained detectable, their integrity was reduced, potentially compromising vaccine effectiveness.

Quantities of S protein cassettes on mRNA (cDNA) and DNA levels in Moderna Spikevax and Pfizer BNT162b2 vaccines

	S protein cassette mRNA (cDNA) (copy number / ml)	S protein cassette DNA (copy number / ml)
Moderna Spikevax	10e9 – 10e10	10e7 – 10e9
Pfizer BNT162b2	10e10 – 10e11	10e8 – 10e9

Table 2: Summarized mRNA and DNA quantities in the Moderna and Pfizer Vials.

Discussion

This research study conducted Molecular analysis of the following lots of Moderna and Pfizer COVID genetic vaccines:

Spikevax (Moderna): MV1013A, 200023A, 200156A, 223049, 200090A, 200106A, 200100A, 3005885, 3005836, 000090A, 000058A, 3005241, 3005697, 3006272, MV1018A, 400012A, 400011A, and

BNT162b2 (Pfizer): FP9632, 1F1051A, 1LO84A, 1F1047A, 1F1059A, 1F1055A, PCB0020.

Analysis of the samples using quantitative multiplex Real-Time PCR revealed:

- Inter-individual heterogeneity between individual lots is remarkable, with a difference in the quantity of declared mRNA of up to 10x.
- All Spikevax (Moderna) and BNT162b2 (Pfizer) lots tested contain a significant admixture of DNA, which is most likely the complete cassette for S protein cloned in an expression vector carrying intact regulatory sequences. Thus, expression of full-length mRNA for S protein from this DNA cassette cannot be excluded.
- The Real-Time PCR measured quantity of DNA in all preparations is comparable to the quantity of the only officially declared item, which is mRNA for S protein. This suggests that it is therefore not DNA “contamination”, but rather a regular admixture, undeclared by either manufacturer.
- Based on the Real-Time PCR measurement of the quantity of DNA present, another degree of heterogeneity can be observed between individual lots, where the possibility that other DNA construct(s), which identity is currently unknown, might also be present in some lots.

The presence of Escherichia coli DNA sequences raises questions about manufacturing quality control. While residual DNA from bacterial propagation systems is not uncommon in recombinant DNA products, its detection in these vaccines suggests incomplete purification during production. Regulatory standards such as those outlined by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) set limits on allowable residual DNA [4,5]. The observed DNA content may exceed these limits and pose theoretical risks related to genomic integration or immunological responses.

Furthermore, the observed variability in mRNA content may affect dose consistency. mRNA vaccines rely on precise nucleic acid delivery to ensure effective antigen expression. Deviations of 20–30% across lots, as observed in this study, may lead to inconsistent immune responses and altered clinical outcomes.

The continued use of the Wuhan strain's S protein sequence in both vaccines highlights a notable concern. Since early 2020, SARS-CoV-2 variants with significantly altered spike protein structures have dominated viral circulation. The use of an outdated sequence may reduce the vaccines' protective efficacy, particularly against newer variants with substantial antigenic drift.

The findings regarding mRNA degradation in expired samples emphasize the importance of strict adherence to storage protocols. Even with ultra-cold storage conditions, prolonged storage beyond designated expiration dates resulted in partial mRNA degradation, potentially impairing vaccine performance.

The above data shows a high degree of heterogeneity between individual Spikevax (Moderna) and BNT162b2 (Pfizer) preparations, measured by the quantity of declared mRNA for S protein. The individual lots differ in the quantity of the target mRNA sequence by 1 order of magnitude (10x).

In all preparations tested, a high quantity of double-stranded DNA (cloning expression vector, DNA cassette for S protein) was identified. Interestingly, the quantity of DNA was comparable to the quantity of mRNA declared by the manufacturer.

Such a high quantity of DNA clearly cannot be considered mere "contamination" during the manufacturing process. In the case of "a contamination", the quantity of the contaminating DNA would be expected many orders of magnitude lower, in the range of approximately 10e2 or 10e3 copies/ml. A random "contamination" of the Spikevax and BNT162b2 preparations with double-stranded DNA during the manufacturing process can therefore be excluded.

Moreover, unremarkable, borderline, contamination with genomic DNA of *Escherichia coli* (GMO used for large-scale production of cloning vectors) was found only in two cases of the BNT162b2 preparation (Pfizer), in individual units of copies of the target microorganism / ml. This might be considered a negligible finding in comparison to the large quantities of DNA of cloning vector and S cassette found in all lots tested. No SV40 was identified.

The presence of such high quantities of DNA in all the lots tested implies that it might not result from some contamination during an inadequate manufacturing process but rather might be considered a regular (though not officially declared) constituent of all lots tested, present in quantities (almost) identical to the quantity of mRNA for the S protein.

Both Pfizer and Moderna identified the only oligonucleotide materials within their vaccines as being mRNA. The presence of double-stranded DNA, or any other DNA, in the Moderna and Pfizer preparations, was not declared by either the manufacturers.

Conclusion

This study highlights key findings regarding the oligonucleotide content of Spikevax (Moderna) and BNT162b2 (Pfizer) COVID-19 vaccines. The presence of these genetic sequences also raises InflammoThrombotic Immunologic Response (ITIR) concerns [1]. While declared mRNA sequences were confirmed, variability in nucleic acid content and the presence of undeclared DNA sequences underscore the need for improved quality control in vaccine manufacturing.

Key recommendations include:

- Enhanced Purification Protocols: Manufacturers should review DNA removal processes to minimize residual contamination and reduce InflammoThrombotic Immunologic Response (ITIR).
- Stricter Lot Testing: Additional oversight is recommended to ensure batch-to-batch consistency in nucleic acid content.
- Genomic Updates: Given the ongoing evolution of SARS-CoV-2 variants, vaccine designs should align with contemporary circulating strains.
- Removal of contaminated genetic vaccines from the market.
- Stricter oversight by regulatory agencies including the FDA, EMA and ŠÚKL.

Continued vigilance in vaccine production and regulatory oversight is crucial to ensuring public confidence in mRNA and DNA vaccine technology and maximizing global immunization efforts.

Funding

This study was carried out in response to a formal request by Slovakian Prime Minister Mr. Robert Fico in and through the office of the Plenipotentiary for the Slovak Republic to assess the nucleic acid content in multiple lots of Spikevax and BNT162b2 vaccines.

IRB: No Institutional Review Board required.

Conflict of Interest

The authors declare no conflict-of-interest.

Data Availability

Further data is available if determined appropriate by submission request including but not limited to the individual(s) and institution(s), purpose/intent of request, and pertinent relevant requested information as deemed applicable by the authors. https://www.flemingmethod.com/

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