**SUPPLEMENTARY MATERIALS**

Table

Description automatically generated

**Figure S1**. **Precision studies using the automated, high-throughput SARS-CoV-2 NGS workflow.** (A) Intra-assay precision of 1,272 variants from 175 specimens repeated 3 times. The mean of the detected variant frequencies (blue dot) and coefficients of variations (orange dot) are shown. (B) Inter-assay precision of 946 variants from 160 specimens replicated 3 times. The mean of the detected variant frequencies (blue dot) and coefficients of variations (orange dot) are shown.

Chart

Description automatically generated

**Figure S2**. Weekly SARS-CoV-2 variant proportions. A. Variant proportions were calculated against total cases sequenced each week. B. Total number of cases sequenced for each week is indicated with scale bar.

**Table S1. Primer pools for the automated, high-throughput SARS-CoV-2 NGS workflow**

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer Name (nCoV-2019\_)** | **Sequence (5' to 3')** | **Pool** | **Ratio1** |
| 1\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACCAACCAACTTTCGATCTCTTGT | 1A | 1X |
| **1\_RIGHTv2** | **GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AAGTGCCATCTTTAAGATGTTGACG** | 1A | 1X |
| 2\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CTGTTTTACAGGTTCGCGACGT | 2A | 1X |
| 2\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAAGGATCAGTGCCAAGCTCGT | 2A | 1X |
| 3\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CGGTAATAAAGGAGCTGGTGGC | 1B | 1X |
| 3\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AAGGTGTCTGCAATTCATAGCTCT | 1B | 1X |
| 4\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGTGTATACTGCTGCCGTGAAC | 2B | 1X |
| 4\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CACAAGTAGTGGCACCTTCTTTAGT | 2B | 1X |
| 5\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGGTGAAACTTCATGGCAGACG | 1A | 1X |
| 5\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATTGATGTTGACTTTCTCTTTTTGGAGT | 1A | 1X |
| 6\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGTGTTGTTGGAGAAGGTTCCG | 2A | 1X |
| 6\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAGCGGCCTTCTGTAAAACACG | 2A | 1X |
| 7\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ATCAGAGGCTGCTCGTGTTGTA | 1B | 1X |
| 7\_LEFT\_alt0 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CATTTGCATCAGAGGCTGCTCG | 1A | 1X |
| 7\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGCACAGGTGACAATTTGTCCA | 1B | 1X |
| 7\_RIGHT\_alt5 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGGTGACAATTTGTCCACCGAC | 1A | 1X |
| 8\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGAGTTTCTTAGAGACGGTTGGGA | 2B | 1X |
| 8\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GCTTCAACAGCTTCACTAGTAGGT | 2B | 1X |
| 9\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCCCACAGAAGTGTTAACAGAGGA | 1B | 1X |
| 9\_LEFT\_alt4 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TTCCCACAGAAGTGTTAACAGAGG | 1A | 1X |
| **9\_RIGHTv2** | **GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTTATGACAGCATCTGCCACAA** | 1B | 1X |
| 9\_RIGHT\_alt2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GACAGCATCTGCCACAACACAG | 1A | 1X |
| 10\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGAGAAGTGCTCTGCCTATACAGT | 2A | 1X |
| 10\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TCATCTAACCAATCTTCTTCTTGCTCT | 2A | 1X |
| 11\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGAATTTGGTGCCACTTCTGCT | 1B | 1X |
| 11\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TCATCAGATTCAACTTGCATGGCA | 1B | 1X |
| 12\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AAACATGGAGGAGGTGTTGCAG | 2B | 1X |
| 12\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTCACTCTTCATTTCCAAAAAGCTTGA | 2B | 1X |
| 13\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCGCACAAATGTCTACTTAGCTGT | 1A | 1X |
| **13\_RIGHTv2** | **GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATAACCACAGCAGTTAAAACAC** | 1A | 1X |
| 14\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CATCCAGATTCTGCCACTCTTGT | 2A | 1X |
| 14\_LEFT\_alt4 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGGCAATCTTCATCCAGATTCTGC | 2B | 1X |
| 14\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGTTTCCACACAGACAGGCATT | 2A | 1X |
| 14\_RIGHT\_alt2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGCGTGTTTCTTCTGCATGTGC | 2B | 1X |
| 15\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACAGTGCTTAAAAAGTGTAAAAGTGCC | 1B | 1X |
| 15\_LEFT\_alt1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGTGCTTAAAAAGTGTAAAAGTGCCT | 1A | 1X |
| **15\_RIGHTv2** | **GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGAAACAGAAACTGTAGCTGGC** | 1B | 1X |
| 15\_RIGHT\_alt3 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACTGTAGCTGGCACTTTGAGAGA | 1A | 1X |
| 16\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AATTTGGAAGAAGCTGCTCGGT | 2A | 1X |
| 16\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CACAACTTGCGTGTGGAGGTTA | 2A | 1X |
| 17\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CTTCTTTCTTTGAGAGAAGTGAGGACT | 1B | 1X |
| 17\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTTGTTGGAGTGTTAACAATGCAGT | 1B | 1X |
| 18\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGGAAATACCCACAAGTTAATGGTTTAAC | 2B | 1X |
| 18\_LEFT\_alt2 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACTTCTATTAAATGGGCAGATAACAACTGT | 2A | 1X |
| 18\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGCTTGTTTACCACACGTACAAGG | 2B | 1X |
| 18\_RIGHT\_alt1 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GCTTGTTTACCACACGTACAAGG | 2A | 1X |
| 19\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GCTGTTATGTACATGGGCACACT | 1A | 1X |
| 19\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGTCCAACTTAGGGTCAATTTCTGT | 1A | 1X |
| 20\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACAAAGAAAACAGTTACACAACAACCA | 2B | 1X |
| 20\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACGTGGCTTTATTAGTTGCATTGTT | 2B | 1X |
| **21\_LEFTv2** | **ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGGTGGCTATTGATTATAAACACTACACA** | 1B | 1X |
| 21\_LEFT\_alt2 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGCTATTGATTATAAACACTACACACCCT | 1A | 1X |
| 21\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAGATCTGTGTGGCCAACCTCT | 1B | 1X |
| 21\_RIGHT\_alt0 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GATCTGTGTGGCCAACCTCTTC | 1A | 1X |
| 22\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACTACCGAAGTTGTAGGAGACATTATACT | 2A | 1X |
| 22\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACAGTATTCTTTGCTATAGTAGTCGGC | 2A | 1X |
| 23\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACAACTACTAACATAGTTACACGGTGT | 1B | 1X |
| 23\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACCAGTACAGTAGGTTGCAATAGTG | 1B | 1X |
| 24\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGGCATGCCTTCTTACTGTACTG | 2B | 1X |
| 24\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACATTCTAACCATAGCTGAAATCGGG | 2B | 1X |
| 25\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GCAATTGTTTTTCAGCTATTTTGCAGT | 1A | 1X |
| 25\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACTGTAGTGACAAGTCTCTCGCA | 1A | 1X |
| 26\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TTGTGATACATTCTGTGCTGGTAGT | 2A, 2B | 1X |
| 26\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TCCGCACTATCACCAACATCAG | 2A, 2B | 1X |
| 27\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACTACAGTCAGCTTATGTGTCAACC | 1B | 1X |
| 27\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AATACAAGCACCAAGGTCACGG | 1B | 1X |
| 28\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACATAGAAGTTACTGGCGATAGTTGT | 2B | 1X |
| 28\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGTTTAGACATGACATGAACAGGTGT | 2B | 1X |
| 29\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACTTGTGTTCCTTTTTGTTGCTGC | 1A, 1B | 1X |
| 29\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGTGTACTCTATAAGTTTTGATGGTGTGT | 1A, 1B | 1X |
| 30\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GCACAACTAATGGTGACTTTTTGCA | 2A | 1X |
| 30\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACCACTAGTAGATACACAAACACCAG | 2A | 1X |
| 31\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TTCTGAGTACTGTAGGCACGGC | 1B | 1X |
| 31\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACAGAATAAACACCAGGTAAGAATGAGT | 1B | 1X |
| 32\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGGTGAATACAGTCATGTAGTTGCC | 2B | 1X |
| **32\_RIGHTv2** | **GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GGTAATAGCACATCACTACGCAAC** | 2B | 1X |
| 33\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACTTTTGAAGAAGCTGCGCTGT | 1A | 1X |
| 33\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGGACAGTAAACTACGTCATCAAGC | 1A | 1X |
| 34\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCCCATCTGGTAAAGTTGAGGGT | 2A | 1X |
| 34\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGTGAAATTGGGCCTCATAGCA | 2A | 1X |
| 35\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGTTCGCATTCAACCAGGACAG | 1B | 1X |
| 35\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACTTCATAGCCACAAGGTTAAAGTCA | 1B | 1X |
| **36\_LEFTv2** | **ACACTCTTTCCCTACACGACGCTCTTCCGATCT ATTACAGTTAATGTTTTAGCTTGGTTGTA** | 2B | 1X |
| 36\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GAACAAAGACCATTGAGTACTCTGGA | 2B | 1X |
| 37\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACACACCACTGGTTGTTACTCAC | 1A | 1X |
| 37\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GTCCACACTCTCCTAGCACCAT | 1A | 1X |
| 38\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACTGTGTTATGTATGCATCAGCTGT | 2A | 1X |
| **38\_RIGHTv2** | **GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATCATAAACACCAAGAGTCAGTCTAAA** | 2A | 1X |
| 39\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGTATTGCCCTATTTTCTTCATAACTGGT | 1B | 1X |
| 39\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGTAACTGGACACATTGAGCCC | 1B | 1X |
| 40\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGCACATCAGTAGTCTTACTCTCAGT | 2B | 1X |
| 40\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CATGGCTGCATCACGGTCAAAT | 2B | 1X |
| 41\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GTTCCCTTCCATCATATGCAGCT | 1A | 1X |
| 41\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGGTATGACAACCATTAGTTTGGCT | 1A | 1X |
| 42\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGCAAGAGATGGTTGTGTTCCC | 2A | 1X |
| 42\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CCTACCTCCCTTTGTTGTGTTGT | 2A | 1X |
| 43\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TACGACAGATGTCTTGTGCTGC | 1B | 1X |
| 43\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGCAGCATCTACAGCAAAAGCA | 1B | 1X |
| 44\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGCCACAGTACGTCTACAAGCT | 2B | 1X |
| 44\_LEFT\_alt3 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CCACAGTACGTCTACAAGCTGG | 2A | 1X |
| 44\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AACCTTTCCACATACCGCAGAC | 2B | 1X |
| 44\_RIGHT\_alt0 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CGCAGACGGTACAGACTGTGTT | 2A | 1X |
| 45\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TACCTACAACTTGTGCTAATGACCC | 1A | 1X |
| 45\_LEFT\_alt2 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGTATGTACAAATACCTACAACTTGTGCT | 1B | 1X |
| 45\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AAATTGTTTCTTCATGTTGGTAGTTAGAGA | 1A | 1X |
| 45\_RIGHT\_alt7 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTCATGTTGGTAGTTAGAGAAAGTGTGTC | 1B | 1X |
| 46\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGTCGCTTCCAAGAAAAGGACG | 2B | 1X |
| 46\_LEFT\_alt1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CGCTTCCAAGAAAAGGACGAAGA | 2A | 1X |
| 46\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CACGTTCACCTAAGTTGGCGTA | 2B | 1X |
| 46\_RIGHT\_alt2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CACGTTCACCTAAGTTGGCGTAT | 2A | 1X |
| 47\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGGACTGGTATGATTTTGTAGAAAACCC | 1A | 1X |
| 47\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AATAACGGTCAAAGAGTTTTAACCTCTC | 1A | 1X |
| 48\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGTTGACACTGACTTAACAAAGCCT | 2B | 1X |
| 48\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAGATTACCAGAAGCAGCGTGC | 2B | 1X |
| 49\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGGAATTACTTGTGTATGCTGCTGA | 1B | 1X |
| 49\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGACGATGACTTGGTTAGCATTAATACA | 1B | 1X |
| 50\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GTTGATAAGTACTTTGATTGTTACGATGGT | 2A | 1X |
| 50\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAACATGTTGTGCCAACCACCA | 2A | 1X |
| 51\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCAATAGCCGCCACTAGAGGAG | 1A | 1X |
| 51\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGTGCATTAACATTGGCCGTGA | 1A | 1X |
| 52\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CATCAGGAGATGCCACAACTGC | 2B | 1X |
| 52\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GTTGAGAGCAAAATTCATGAGGTCC | 2B | 1X |
| 53\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGCAAAATGTTGGACTGAGACTGA | 1B | 1X |
| 53\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGCCTCATAAAACTCAGGTTCCC | 1B | 1X |
| 54\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGAGTTAACAGGACACATGTTAGACA | 2A | 1X |
| 54\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AACCAAAAACTTGTCCATTAGCACA | 2A | 1X |
| 55\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACTCAACTTTACTTAGGAGGTATGAGCT | 1A | 1X |
| 55\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GGTGTACTCTCCTATTTGTACTTTACTGT | 1A | 1X |
| 56\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACCTAGACCACCACTTAACCGA | 2B | 1X |
| 56\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACACTATGCGAGCAGAAGGGTA | 2B | 1X |
| 57\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ATTCTACACTCCAGGGACCACC | 1B | 1X |
| 57\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GTAATTGAGCAGGGTCGCCAAT | 1B | 1X |
| 58\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGATTTGAGTGTTGTCAATGCCAGA | 2A | 1X |
| 58\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CTTTTCTCCAAGCAGGGTTACGT | 2A | 1X |
| 59\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCACGCATGATGTTTCATCTGCA | 1A | 1X |
| 59\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AAGAGTCCTGTTACATTTTCAGCTTG | 1A | 1X |
| 60\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGATAGAGACCTTTATGACAAGTTGCA | 2B | 1X |
| 60\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GGTACCAACAGCTTCTCTAGTAGC | 2B | 1X |
| 61\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGTTTATCACCCGCGAAGAAGC | 1B | 1X |
| 61\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATCACATAGACAACAGGTGCGC | 1B | 1X |
| 62\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGCACATGGCTTTGAGTTGACA | 2A | 1X |
| 62\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GTTGAACCTTTCTACAAGCCGC | 2A | 1X |
| 63\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGTTAAGCGTGTTGACTGGACT | 1A | 1X |
| 63\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACAAACTGCCACCATCACAACC | 1A | 1X |
| 64\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCGATAGATATCCTGCTAATTCCATTGT | 2B | 1X |
| 64\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGTCTTGTAAAAGTGTTCCAGAGGT | 2B | 1X |
| 65\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GCTGGCTTTAGCTTGTGGGTTT | 1B | 1X |
| 65\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGTCAGTCATAGAACAAACACCAATAGT | 1B | 1X |
| 66\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGGTGTGGACATTGCTGCTAAT | 2A | 1X |
| 66\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TCAATTTCCATTTGACTCCTGGGT | 2A | 1X |
| 67\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GTTGTCCAACAATTACCTGAAACTTACT | 1A, 1B | 1X |
| 67\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CAACCTTAGAAACTACAGATAAATCTTGGG | 1A, 1B | 1X |
| 68\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACAGGTTCATCTAAGTGTGTGTGT | 2A, 2B | 1X |
| 68\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CTCCTTTATCAGAACCAGCACCA | 2A, 2B | 1X |
| 69\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGTCGCAAAATATACTCAACTGTGTCA | 1B | 1X |
| 69\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TCTTTATAGCCACGGAACCTCCA | 1B | 1X |
| 70\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACAAAAGAAAATGACTCTAAAGAGGGTTT | 2B | 1X |
| 70\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGACCTTCTTTTAAAGACATAACAGCAG | 2B | 1X |
| 71\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACAAATCCAATTCAGTTGTCTTCCTATTC | 1A | 1X |
| 71\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGGAAAAGAAAGGTAAGAACAAGTCCT | 1A | 1X |
| 72\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACACGTGGTGTTTATTACCCTGAC | 2B | 1X |
| 72\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACTCTGAACTCACTTTCCATCCAAC | 2B | 1X |
| 73\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CAATTTTGTAATGATCCATTTTTGGGTGT | 1B | 1X |
| 73\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CACCAGCTGTCCAACCTGAAGA | 1B | 1X |
| 74\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACATCACTAGGTTTCAAACTTTACTTGC | 2A, 2B | 2X |
| 74\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GCAACACAGTTGCTGATTCTCTTC | 2A, 2B | 2X |
| 75\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGAGTCCAACCAACAGAATCTATTGT | 1A, 1B | 1X |
| 75\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACCACCAACCTTAGAATCAAGATTGT | 1A, 1B | 1X |
| 76\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AGGGCAAACTGGAAAGATTGCT | 2B | 1X |
| 76\_LEFT\_alt3 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGGCAAACTGGAAAGATTGCTGA | 2A | 1X |
| **76\_RIGHTv2** | **GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TCTCTGCCAAATTGTTGGAAAGGCA** | 2B | 1X |
| 76\_RIGHT\_alt0 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACCTGTGCCTGTTAAACCATTGA | 2A | 1X |
| 77\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CCAGCAACTGTTTGTGGACCTA | 1B | 1X |
| 77\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CAGCCCCTATTAAACAGCCTGC | 1B | 1X |
| 78\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CAACTTACTCCTACTTGGCGTGT | 2B | 1X |
| 78\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGTGTACAAAAACTGCCATATTGCA | 2B | 1X |
| 79\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GTGGTGATTCAACTGAATGCAGC | 1A, 1B | 1X |
| 79\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CATTTCATCTGTGAGCAAAGGTGG | 1A, 1B | 1X |
| 80\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TTGCCTTGGTGATATTGCTGCT | 2A | 1X |
| 80\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGGAGCTAAGTTGTTTAACAAGCG | 2A | 1X |
| 81\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GCACTTGGAAAACTTCAAGATGTGG | 1B | 1X |
| 81\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GTGAAGTTCTTTTCTTGTGCAGGG | 1B | 1X |
| 82\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGGCTATCATCTTATGTCCTTCCCT | 2B | 1X |
| 82\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGCCAGAGATGTCACCTAAATCAA | 2B | 1X |
| 83\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCCTTTGCAACCTGAATTAGACTCA | 1A | 1X |
| 83\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTTGACTCCTTTGAGCACTGGC | 1A | 1X |
| 84\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGCTGTAGTTGTCTCAAGGGCT | 2A | 1X |
| 84\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGGTGTGAGTAAACTGTTACAAACAAC | 2A | 1X |
| 85\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACTAGCACTCTCCAAGGGTGTT | 1B | 1X |
| 85\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACACAGTCTTTTACTCCAGATTCCC | 1B | 1X |
| 86\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCAGGTGATGGCACAACAAGTC | 2B | 1X |
| 86\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACGAAAGCAAGAAAAAGAAGTACGC | 2B | 1X |
| 87\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CGACTACTAGCGTGCCTTTGTA | 1A | 1X |
| 87\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACTAGGTTCCATTGTTCAAGGAGC | 1A | 1X |
| 88\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CCATGGCAGATTCCAACGGTAC | 2A | 1X |
| 88\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGGTCAGAATAGTGCCATGGAGT | 2A | 1X |
| **89\_LEFTv2** | **ACACTCTTTCCCTACACGACGCTCTTCCGATCT CGCGTACGCGTTCCATGTGGTC** | 1B | 1X |
| 89\_LEFT\_alt2 | ACACTCTTTCCCTACACGACGCTCTTCCGATCT CGCGTTCCATGTGGTCATTCAA | 1A | 1X |
| **89\_RIGHTv2** | **GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATAGTAACCTGAAAGTCAACGAGAT** | 1B | 1X |
| 89\_RIGHT\_alt4 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACGAGATGAAACATCTGTTGTCACT | 1A | 1X |
| 90\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT ACACAGACCATTCCAGTAGCAGT | 2B | 1X |
| 90\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGAAATGGTGAATTGCCCTCGT | 2B | 1X |
| 91\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCACTACCAAGAGTGTGTTAGAGGT | 1B | 1X |
| 91\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTCAAGTGAGAACCAAAAGATAATAAGCA | 1B | 1X |
| 92\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TTTGTGCTTTTTAGCCTTTCTGCT | 2A | 1X |
| 92\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGGTTCCTGGCAATTAATTGTAAAAGG | 2A | 1X |
| 93\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGAGGCTGGTTCTAAATCACCCA | 1A | 1X |
| 93\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AGGTCTTCCTTGCCATGTTGAG | 1A | 1X |
| 94\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGCCCCAAGGTTTACCCAATAA | 2B | 1X |
| 94\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTTGGCAATGTTGTTCCTTGAGG | 2B | 1X |
| 95\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGAGGGAGCCTTGAATACACCA | 1B | 1X |
| 95\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CAGTACGTTTTTGCCGAGGCTT | 1B | 1X |
| 96\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT GCCAACAACAACAAGGCCAAAC | 2A | 1X |
| 96\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAGGCTCTGTTGGTGGGAATGT | 2A | 1X |
| 97\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGGATGACAAAGATCCAAATTTCAAAGA | 1A | 1X |
| 97\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT ACACACTGATTAAAGATTGCTATGTGAG | 1A | 1X |
| 98\_LEFT | ACACTCTTTCCCTACACGACGCTCTTCCGATCT AACAATTGCAACAATCCATGAGCA | 2B | 1X |
| 98\_RIGHT | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTCTCCTAAGAAGCTATTAAAATCACATGG | 2B | 1X |

**1** Relative primer concentration in each pool is indicated under Ratio.

SARS-CoV-2 specific sequences are from the ARTICv3 primer set (Quick 2020).

Primers from Itokawa et al (2020) are marked in bold.

Quick J, Loman NJ. hCoV-2019/nCoV-2019 Version 3 Amplicon Set. March 24, 2020. Available: <https://artic.network/resources/ncov/ncov-amplicon-v3.pdf>.

Itokawa K, Sekizuka T, Hashino M, Tanaka R, Kuroda M (2020) Disentangling primer interactions improves SARS-CoV-2 genome sequencing by multiplex tiling PCR. PLoS ONE 15(9): e0239403.

**Table S2. Modified primer concentration for each pool for a standard ARTIC v3 workflow**

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer Name (nCoV-2019\_)** | **Sequence (5' to 3')** | **Pool** | **Ratio1** |
| 1\_LEFT | ACCAACCAACTTTCGATCTCTTGT | 1 | 1X |
| 1\_RIGHT | CATCTTTAAGATGTTGACGTGCCTC | 1 | 1X |
| 2\_LEFT | CTGTTTTACAGGTTCGCGACGT | 2 | 1X |
| 2\_RIGHT | TAAGGATCAGTGCCAAGCTCGT | 2 | 1X |
| 3\_LEFT | CGGTAATAAAGGAGCTGGTGGC | 1 | 1X |
| 3\_RIGHT | AAGGTGTCTGCAATTCATAGCTCT | 1 | 1X |
| 4\_LEFT | GGTGTATACTGCTGCCGTGAAC | 2 | 1X |
| 4\_RIGHT | CACAAGTAGTGGCACCTTCTTTAGT | 2 | 1X |
| 5\_LEFT | TGGTGAAACTTCATGGCAGACG | 1 | 7X |
| 5\_RIGHT | ATTGATGTTGACTTTCTCTTTTTGGAGT | 1 | 7X |
| 6\_LEFT | GGTGTTGTTGGAGAAGGTTCCG | 2 | 2.5X |
| 6\_RIGHT | TAGCGGCCTTCTGTAAAACACG | 2 | 2.5X |
| 7\_LEFT | ATCAGAGGCTGCTCGTGTTGTA | 1 | 1X |
| 7\_LEFT\_alt0 | CATTTGCATCAGAGGCTGCTCG | 1 | 1X |
| 7\_RIGHT | TGCACAGGTGACAATTTGTCCA | 1 | 1X |
| 7\_RIGHT\_alt5 | AGGTGACAATTTGTCCACCGAC | 1 | 1X |
| 8\_LEFT | AGAGTTTCTTAGAGACGGTTGGGA | 2 | 1X |
| 8\_RIGHT | GCTTCAACAGCTTCACTAGTAGGT | 2 | 1X |
| 9\_LEFT | TCCCACAGAAGTGTTAACAGAGGA | 1 | 1X |
| 9\_LEFT\_alt4 | TTCCCACAGAAGTGTTAACAGAGG | 1 | 1X |
| 9\_RIGHT | ATGACAGCATCTGCCACAACAC | 1 | 1X |
| 9\_RIGHT\_alt2 | GACAGCATCTGCCACAACACAG | 1 | 1X |
| 10\_LEFT | TGAGAAGTGCTCTGCCTATACAGT | 2 | 1X |
| 10\_RIGHT | TCATCTAACCAATCTTCTTCTTGCTCT | 2 | 1X |
| 11\_LEFT | GGAATTTGGTGCCACTTCTGCT | 1 | 1X |
| 11\_RIGHT | TCATCAGATTCAACTTGCATGGCA | 1 | 1X |
| 12\_LEFT | AAACATGGAGGAGGTGTTGCAG | 2 | 7X |
| 12\_RIGHT | TTCACTCTTCATTTCCAAAAAGCTTGA | 2 | 7X |
| 13\_LEFT | TCGCACAAATGTCTACTTAGCTGT | 1 | 7X |
| 13\_RIGHT | ACCACAGCAGTTAAAACACCCT | 1 | 7X |
| 14\_LEFT | CATCCAGATTCTGCCACTCTTGT | 2 | 1X |
| 14\_LEFT\_alt4 | TGGCAATCTTCATCCAGATTCTGC | 2 | 1X |
| 14\_RIGHT | AGTTTCCACACAGACAGGCATT | 2 | 1X |
| 14\_RIGHT\_alt2 | TGCGTGTTTCTTCTGCATGTGC | 2 | 1X |
| 15\_LEFT | ACAGTGCTTAAAAAGTGTAAAAGTGCC | 1 | 1X |
| 15\_LEFT\_alt1 | AGTGCTTAAAAAGTGTAAAAGTGCCT | 1 | 1X |
| 15\_RIGHT | AACAGAAACTGTAGCTGGCACT | 1 | 1X |
| 15\_RIGHT\_alt3 | ACTGTAGCTGGCACTTTGAGAGA | 1 | 1X |
| 16\_LEFT | AATTTGGAAGAAGCTGCTCGGT | 2 | 1X |
| 16\_RIGHT | CACAACTTGCGTGTGGAGGTTA | 2 | 1X |
| 17\_LEFT | CTTCTTTCTTTGAGAGAAGTGAGGACT | 1 | 7X |
| 17\_RIGHT | TTTGTTGGAGTGTTAACAATGCAGT | 1 | 7X |
| 18\_LEFT | TGGAAATACCCACAAGTTAATGGTTTAAC | 2 | 1X |
| 18\_LEFT\_alt2 | ACTTCTATTAAATGGGCAGATAACAACTGT | 2 | 1X |
| 18\_RIGHT | AGCTTGTTTACCACACGTACAAGG | 2 | 1X |
| 18\_RIGHT\_alt1 | GCTTGTTTACCACACGTACAAGG | 2 | 1X |
| 19\_LEFT | GCTGTTATGTACATGGGCACACT | 1 | 2.5X |
| 19\_RIGHT | TGTCCAACTTAGGGTCAATTTCTGT | 1 | 2.5X |
| 20\_LEFT | ACAAAGAAAACAGTTACACAACAACCA | 2 | 1X |
| 20\_RIGHT | ACGTGGCTTTATTAGTTGCATTGTT | 2 | 1X |
| 21\_LEFT | TGGCTATTGATTATAAACACTACACACCC | 1 | 7X |
| 21\_LEFT\_alt2 | GGCTATTGATTATAAACACTACACACCCT | 1 | 7X |
| 21\_RIGHT | TAGATCTGTGTGGCCAACCTCT | 1 | 7X |
| 21\_RIGHT\_alt0 | GATCTGTGTGGCCAACCTCTTC | 1 | 7X |
| 22\_LEFT | ACTACCGAAGTTGTAGGAGACATTATACT | 2 | 1X |
| 22\_RIGHT | ACAGTATTCTTTGCTATAGTAGTCGGC | 2 | 1X |
| 23\_LEFT | ACAACTACTAACATAGTTACACGGTGT | 1 | 7X |
| 23\_RIGHT | ACCAGTACAGTAGGTTGCAATAGTG | 1 | 7X |
| 24\_LEFT | AGGCATGCCTTCTTACTGTACTG | 2 | 1X |
| 24\_RIGHT | ACATTCTAACCATAGCTGAAATCGGG | 2 | 1X |
| 25\_LEFT | GCAATTGTTTTTCAGCTATTTTGCAGT | 1 | 1X |
| 25\_RIGHT | ACTGTAGTGACAAGTCTCTCGCA | 1 | 1X |
| 26\_LEFT | TTGTGATACATTCTGTGCTGGTAGT | 2 | 7X |
| 26\_RIGHT | TCCGCACTATCACCAACATCAG | 2 | 7X |
| 27\_LEFT | ACTACAGTCAGCTTATGTGTCAACC | 1 | 1X |
| 27\_RIGHT | AATACAAGCACCAAGGTCACGG | 1 | 1X |
| 28\_LEFT | ACATAGAAGTTACTGGCGATAGTTGT | 2 | 2.5X |
| 28\_RIGHT | TGTTTAGACATGACATGAACAGGTGT | 2 | 2.5X |
| 29\_LEFT | ACTTGTGTTCCTTTTTGTTGCTGC | 1 | 2.5X |
| 29\_RIGHT | AGTGTACTCTATAAGTTTTGATGGTGTGT | 1 | 2.5X |
| 30\_LEFT | GCACAACTAATGGTGACTTTTTGCA | 2 | 1X |
| 30\_RIGHT | ACCACTAGTAGATACACAAACACCAG | 2 | 1X |
| 31\_LEFT | TTCTGAGTACTGTAGGCACGGC | 1 | 1X |
| 31\_RIGHT | ACAGAATAAACACCAGGTAAGAATGAGT | 1 | 1X |
| 32\_LEFT | TGGTGAATACAGTCATGTAGTTGCC | 2 | 1X |
| 32\_RIGHT | AGCACATCACTACGCAACTTTAGA | 2 | 1X |
| 33\_LEFT | ACTTTTGAAGAAGCTGCGCTGT | 1 | 1X |
| 33\_RIGHT | TGGACAGTAAACTACGTCATCAAGC | 1 | 1X |
| 34\_LEFT | TCCCATCTGGTAAAGTTGAGGGT | 2 | 1X |
| 34\_RIGHT | AGTGAAATTGGGCCTCATAGCA | 2 | 1X |
| 35\_LEFT | TGTTCGCATTCAACCAGGACAG | 1 | 1X |
| 35\_RIGHT | ACTTCATAGCCACAAGGTTAAAGTCA | 1 | 1X |
| 36\_LEFT | TTAGCTTGGTTGTACGCTGCTG | 2 | 2.5X |
| 36\_RIGHT | GAACAAAGACCATTGAGTACTCTGGA | 2 | 2.5X |
| 37\_LEFT | ACACACCACTGGTTGTTACTCAC | 1 | 1X |
| 37\_RIGHT | GTCCACACTCTCCTAGCACCAT | 1 | 1X |
| 38\_LEFT | ACTGTGTTATGTATGCATCAGCTGT | 2 | 2.5X |
| 38\_RIGHT | CACCAAGAGTCAGTCTAAAGTAGCG | 2 | 2.5X |
| 39\_LEFT | AGTATTGCCCTATTTTCTTCATAACTGGT | 1 | 1X |
| 39\_RIGHT | TGTAACTGGACACATTGAGCCC | 1 | 1X |
| 40\_LEFT | TGCACATCAGTAGTCTTACTCTCAGT | 2 | 1X |
| 40\_RIGHT | CATGGCTGCATCACGGTCAAAT | 2 | 1X |
| 41\_LEFT | GTTCCCTTCCATCATATGCAGCT | 1 | 1X |
| 41\_RIGHT | TGGTATGACAACCATTAGTTTGGCT | 1 | 1X |
| 42\_LEFT | TGCAAGAGATGGTTGTGTTCCC | 2 | 1X |
| 42\_RIGHT | CCTACCTCCCTTTGTTGTGTTGT | 2 | 1X |
| 43\_LEFT | TACGACAGATGTCTTGTGCTGC | 1 | 1X |
| 43\_RIGHT | AGCAGCATCTACAGCAAAAGCA | 1 | 1X |
| 44\_LEFT | TGCCACAGTACGTCTACAAGCT | 2 | 1X |
| 44\_LEFT\_alt3 | CCACAGTACGTCTACAAGCTGG | 2 | 1X |
| 44\_RIGHT | AACCTTTCCACATACCGCAGAC | 2 | 1X |
| 44\_RIGHT\_alt0 | CGCAGACGGTACAGACTGTGTT | 2 | 1X |
| 45\_LEFT | TACCTACAACTTGTGCTAATGACCC | 1 | 1X |
| 45\_LEFT\_alt2 | AGTATGTACAAATACCTACAACTTGTGCT | 1 | 1X |
| 45\_RIGHT | AAATTGTTTCTTCATGTTGGTAGTTAGAGA | 1 | 1X |
| 45\_RIGHT\_alt7 | TTCATGTTGGTAGTTAGAGAAAGTGTGTC | 1 | 1X |
| 46\_LEFT | TGTCGCTTCCAAGAAAAGGACG | 2 | 1X |
| 46\_LEFT\_alt1 | CGCTTCCAAGAAAAGGACGAAGA | 2 | 1X |
| 46\_RIGHT | CACGTTCACCTAAGTTGGCGTA | 2 | 1X |
| 46\_RIGHT\_alt2 | CACGTTCACCTAAGTTGGCGTAT | 2 | 1X |
| 47\_LEFT | AGGACTGGTATGATTTTGTAGAAAACCC | 1 | 1X |
| 47\_RIGHT | AATAACGGTCAAAGAGTTTTAACCTCTC | 1 | 1X |
| 48\_LEFT | TGTTGACACTGACTTAACAAAGCCT | 2 | 1X |
| 48\_RIGHT | TAGATTACCAGAAGCAGCGTGC | 2 | 1X |
| 49\_LEFT | AGGAATTACTTGTGTATGCTGCTGA | 1 | 1X |
| 49\_RIGHT | TGACGATGACTTGGTTAGCATTAATACA | 1 | 1X |
| 50\_LEFT | GTTGATAAGTACTTTGATTGTTACGATGGT | 2 | 1X |
| 50\_RIGHT | TAACATGTTGTGCCAACCACCA | 2 | 1X |
| 51\_LEFT | TCAATAGCCGCCACTAGAGGAG | 1 | 1X |
| 51\_RIGHT | AGTGCATTAACATTGGCCGTGA | 1 | 1X |
| 52\_LEFT | CATCAGGAGATGCCACAACTGC | 2 | 1X |
| 52\_RIGHT | GTTGAGAGCAAAATTCATGAGGTCC | 2 | 1X |
| 53\_LEFT | AGCAAAATGTTGGACTGAGACTGA | 1 | 1X |
| 53\_RIGHT | AGCCTCATAAAACTCAGGTTCCC | 1 | 1X |
| 54\_LEFT | TGAGTTAACAGGACACATGTTAGACA | 2 | 1X |
| 54\_RIGHT | AACCAAAAACTTGTCCATTAGCACA | 2 | 1X |
| 55\_LEFT | ACTCAACTTTACTTAGGAGGTATGAGCT | 1 | 1X |
| 55\_RIGHT | GGTGTACTCTCCTATTTGTACTTTACTGT | 1 | 1X |
| 56\_LEFT | ACCTAGACCACCACTTAACCGA | 2 | 1X |
| 56\_RIGHT | ACACTATGCGAGCAGAAGGGTA | 2 | 1X |
| 57\_LEFT | ATTCTACACTCCAGGGACCACC | 1 | 1X |
| 57\_RIGHT | GTAATTGAGCAGGGTCGCCAAT | 1 | 1X |
| 58\_LEFT | TGATTTGAGTGTTGTCAATGCCAGA | 2 | 1X |
| 58\_RIGHT | CTTTTCTCCAAGCAGGGTTACGT | 2 | 1X |
| 59\_LEFT | TCACGCATGATGTTTCATCTGCA | 1 | 1X |
| 59\_RIGHT | AAGAGTCCTGTTACATTTTCAGCTTG | 1 | 1X |
| 60\_LEFT | TGATAGAGACCTTTATGACAAGTTGCA | 2 | 1X |
| 60\_RIGHT | GGTACCAACAGCTTCTCTAGTAGC | 2 | 1X |
| 61\_LEFT | TGTTTATCACCCGCGAAGAAGC | 1 | 1X |
| 61\_RIGHT | ATCACATAGACAACAGGTGCGC | 1 | 1X |
| 62\_LEFT | GGCACATGGCTTTGAGTTGACA | 2 | 1X |
| 62\_RIGHT | GTTGAACCTTTCTACAAGCCGC | 2 | 1X |
| 63\_LEFT | TGTTAAGCGTGTTGACTGGACT | 1 | 1X |
| 63\_RIGHT | ACAAACTGCCACCATCACAACC | 1 | 1X |
| 64\_LEFT | TCGATAGATATCCTGCTAATTCCATTGT | 2 | 7X |
| 64\_RIGHT | AGTCTTGTAAAAGTGTTCCAGAGGT | 2 | 7X |
| 65\_LEFT | GCTGGCTTTAGCTTGTGGGTTT | 1 | 1X |
| 65\_RIGHT | TGTCAGTCATAGAACAAACACCAATAGT | 1 | 1X |
| 66\_LEFT | GGGTGTGGACATTGCTGCTAAT | 2 | 7X |
| 66\_RIGHT | TCAATTTCCATTTGACTCCTGGGT | 2 | 7X |
| 67\_LEFT | GTTGTCCAACAATTACCTGAAACTTACT | 1 | 1X |
| 67\_RIGHT | CAACCTTAGAAACTACAGATAAATCTTGGG | 1 | 1X |
| 68\_LEFT | ACAGGTTCATCTAAGTGTGTGTGT | 2 | 1X |
| 68\_RIGHT | CTCCTTTATCAGAACCAGCACCA | 2 | 1X |
| 69\_LEFT | TGTCGCAAAATATACTCAACTGTGTCA | 1 | 1X |
| 69\_RIGHT | TCTTTATAGCCACGGAACCTCCA | 1 | 1X |
| 70\_LEFT | ACAAAAGAAAATGACTCTAAAGAGGGTTT | 2 | 7X |
| 70\_RIGHT | TGACCTTCTTTTAAAGACATAACAGCAG | 2 | 7X |
| 71\_LEFT | ACAAATCCAATTCAGTTGTCTTCCTATTC | 1 | 7X |
| 71\_RIGHT | TGGAAAAGAAAGGTAAGAACAAGTCCT | 1 | 7X |
| 72\_LEFT | ACACGTGGTGTTTATTACCCTGAC | 2 | 1X |
| 72\_RIGHT | ACTCTGAACTCACTTTCCATCCAAC | 2 | 1X |
| 73\_LEFT | CAATTTTGTAATGATCCATTTTTGGGTGT | 1 | 2.5X |
| 73\_RIGHT | CACCAGCTGTCCAACCTGAAGA | 1 | 2.5X |
| 74\_LEFT | ACATCACTAGGTTTCAAACTTTACTTGC | 2 | 7X |
| 74\_RIGHT | GCAACACAGTTGCTGATTCTCTTC | 2 | 7X |
| 75\_LEFT | AGAGTCCAACCAACAGAATCTATTGT | 1 | 7X |
| 75\_RIGHT | ACCACCAACCTTAGAATCAAGATTGT | 1 | 7X |
| 76\_LEFT | AGGGCAAACTGGAAAGATTGCT | 2 | 2.5X |
| 76\_LEFT\_alt3 | GGGCAAACTGGAAAGATTGCTGA | 2 | 2.5X |
| 76\_RIGHT | ACACCTGTGCCTGTTAAACCAT | 2 | 2.5X |
| 76\_RIGHT\_alt0 | ACCTGTGCCTGTTAAACCATTGA | 2 | 2.5X |
| 77\_LEFT | CCAGCAACTGTTTGTGGACCTA | 1 | 1X |
| 77\_RIGHT | CAGCCCCTATTAAACAGCCTGC | 1 | 1X |
| 78\_LEFT | CAACTTACTCCTACTTGGCGTGT | 2 | 1X |
| 78\_RIGHT | TGTGTACAAAAACTGCCATATTGCA | 2 | 1X |
| 79\_LEFT | GTGGTGATTCAACTGAATGCAGC | 1 | 1X |
| 79\_RIGHT | CATTTCATCTGTGAGCAAAGGTGG | 1 | 1X |
| 80\_LEFT | TTGCCTTGGTGATATTGCTGCT | 2 | 1X |
| 80\_RIGHT | TGGAGCTAAGTTGTTTAACAAGCG | 2 | 1X |
| 81\_LEFT | GCACTTGGAAAACTTCAAGATGTGG | 1 | 2.5X |
| 81\_RIGHT | GTGAAGTTCTTTTCTTGTGCAGGG | 1 | 2.5X |
| 82\_LEFT | GGGCTATCATCTTATGTCCTTCCCT | 2 | 1X |
| 82\_RIGHT | TGCCAGAGATGTCACCTAAATCAA | 2 | 1X |
| 83\_LEFT | TCCTTTGCAACCTGAATTAGACTCA | 1 | 2.5X |
| 83\_RIGHT | TTTGACTCCTTTGAGCACTGGC | 1 | 2.5X |
| 84\_LEFT | TGCTGTAGTTGTCTCAAGGGCT | 2 | 1X |
| 84\_RIGHT | AGGTGTGAGTAAACTGTTACAAACAAC | 2 | 1X |
| 85\_LEFT | ACTAGCACTCTCCAAGGGTGTT | 1 | 2.5X |
| 85\_RIGHT | ACACAGTCTTTTACTCCAGATTCCC | 1 | 2.5X |
| 86\_LEFT | TCAGGTGATGGCACAACAAGTC | 2 | 2.5X |
| 86\_RIGHT | ACGAAAGCAAGAAAAAGAAGTACGC | 2 | 2.5X |
| 87\_LEFT | CGACTACTAGCGTGCCTTTGTA | 1 | 1X |
| 87\_RIGHT | ACTAGGTTCCATTGTTCAAGGAGC | 1 | 1X |
| 88\_LEFT | CCATGGCAGATTCCAACGGTAC | 2 | 1X |
| 88\_RIGHT | TGGTCAGAATAGTGCCATGGAGT | 2 | 1X |
| 89\_LEFT | GTACGCGTTCCATGTGGTCATT | 1 | 2.5X |
| 89\_LEFT\_alt2 | CGCGTTCCATGTGGTCATTCAA | 1 | 2.5X |
| 89\_RIGHT | ACCTGAAAGTCAACGAGATGAAACA | 1 | 2.5X |
| 89\_RIGHT\_alt4 | ACGAGATGAAACATCTGTTGTCACT | 1 | 2.5X |
| 90\_LEFT | ACACAGACCATTCCAGTAGCAGT | 2 | 1X |
| 90\_RIGHT | TGAAATGGTGAATTGCCCTCGT | 2 | 1X |
| 91\_LEFT | TCACTACCAAGAGTGTGTTAGAGGT | 1 | 7X |
| 91\_RIGHT | TTCAAGTGAGAACCAAAAGATAATAAGCA | 1 | 7X |
| 92\_LEFT | TTTGTGCTTTTTAGCCTTTCTGCT | 2 | 1X |
| 92\_RIGHT | AGGTTCCTGGCAATTAATTGTAAAAGG | 2 | 1X |
| 93\_LEFT | TGAGGCTGGTTCTAAATCACCCA | 1 | 1X |
| 93\_RIGHT | AGGTCTTCCTTGCCATGTTGAG | 1 | 1X |
| 94\_LEFT | GGCCCCAAGGTTTACCCAATAA | 2 | 1X |
| 94\_RIGHT | TTTGGCAATGTTGTTCCTTGAGG | 2 | 1X |
| 95\_LEFT | TGAGGGAGCCTTGAATACACCA | 1 | 7X |
| 95\_RIGHT | CAGTACGTTTTTGCCGAGGCTT | 1 | 7X |
| 96\_LEFT | GCCAACAACAACAAGGCCAAAC | 2 | 1X |
| 96\_RIGHT | TAGGCTCTGTTGGTGGGAATGT | 2 | 1X |
| 97\_LEFT | TGGATGACAAAGATCCAAATTTCAAAGA | 1 | 7X |
| 97\_RIGHT | ACACACTGATTAAAGATTGCTATGTGAG | 1 | 7X |
| 98\_LEFT | AACAATTGCAACAATCCATGAGCA | 2 | 2.5X |
| 98\_RIGHT | TTCTCCTAAGAAGCTATTAAAATCACATGG | 2 | 2.5X |

Primer sequences are from the ARTICv3 primer set (Quick 2020).

1 Relative primer concentration in each pool is indicated under Ratio.

Quick J, Loman NJ. hCoV-2019/nCoV-2019 Version 3 Amplicon Set. March 24, 2020. Available: <https://artic.network/resources/ncov/ncov-amplicon-v3.pdf>.

**Table S3. SARS-CoV-2 primer binding site mutations found in samples (n=3,506) collected between January and February of 2021**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position**1 | **Reference**  **>Variant** | **Prevalence  in the study cohort, %**2 | **ARTICv3  Primer Name** | **Primer binding site mutation**3 **(5' to 3')** |
| 1,578 | T>C | 1.3 | nCoV-2019\_6\_LEFT | GGTGcTGTTGGAGAAGGTTCCG |
| 2,258 | G>A | 2.7 | nCoV-2019\_7\_RIGHT | TGCACAGGTGAtAATTTGTCCA |
| 2,258 | G>A | 2.7 | nCoV-2019\_7\_RIGHT\_alt5 | AGGTGAtAATTTGTCCACCGAC |
| 4,683 | C>T | 1.0 | nCoV-2019\_15\_RIGHT\_alt3 | ACTGTAaCTGGCACTTTGAGAGA |
| 5,011 | A>C | 1.7 | nCoV-2019\_16\_RIGHT | CACAACgTGCGTGTGGAGGTTA |
| 5,869 | C>T | 2.5 | nCoV-2019\_20\_LEFT | AtAAAGAAAACAGTTACACAACAACCA |
| 6,730 | C>T | 1.0 | nCoV-2019\_23\_LEFT | ACAACTACTAAtATAGTTACACGGTGT |
| 8,264 | G>T | 1.7 | nCoV-2019\_28\_LEFT | ACATAGAAGTTACTtGCGATAGTTGT |
| 9,204 | A>G | 2.7 | nCoV-2019\_31\_LEFT | TTCTGAGTACTGTAGGCACGGC |
| 10,369 | C>T | 1.3 | nCoV-2019\_35\_LEFT | TGTTCGtATTCAACCAGGACAG |
| 10,741 | C>T | 2.3 | nCoV-2019\_35\_RIGHT | ACTTCATAGCCACAAGGTTAAAaTCA |
| 12,484 | C>T | 1.4 | nCoV-2019\_41\_RIGHT | TGGTATaACAACCATTAGTTTGGCT |
| 12,789 | C>T | 4.2 | nCoV-2019\_42\_RIGHT | CCTACCTCCCTTTaTTGTGTTGT |
| 13,019 | C>T | 2.3 | nCoV-2019\_44\_LEFT | TGCCACAGTACGTtTACAAGCT |
| 13,019 | C>T | 2.3 | nCoV-2019\_44\_LEFT\_alt3 | CCACAGTACGTtTACAAGCTGG |
| 16,500 | A>C | 1.3 | nCoV-2019\_54\_RIGHT | AACCAAAAACgTGTCCATTAGCACA |
| 21,364 | C>T | 1.9 | nCoV-2019\_71\_LEFT | ACAAATtCAATTCAGTTGTCTTCCTATTC |
| 22,018 | G>T | 17.7 | nCoV-2019\_72\_RIGHT | ACTCTGAACTCACTTTCCATaCAAC |
| 22,335 | G>T | 1.9 | nCoV-2019\_73\_RIGHT | CACCAGCTGTCaAACCTGAAGA |
| 23,191 | C>T | 1.4 | nCoV-2019\_76\_RIGHT | ACACCTGTGCCTGTTAAACCAT |
| 25,907 | G>T | 41.6 | nCoV-2019\_86\_LEFT | TCAGtTGATGGCACAACAAGTC |
| 26,305 | C>G | 1.3 | nCoV-2019\_86\_RIGHT | ACGAAAGCAAcAAAAAGAAGTACGC |
| 28,087 | C>T | 2.7 | nCoV-2019\_93\_LEFT | TGAGGtTGGTTCTAAATCACCCA |
| 29,362 | C>T | 17.9 | nCoV-2019\_96\_RIGHT | TAGGCTCTGTTGGTGGaAATGT |
| 29,377 | T>A | 1.5 | nCoV-2019\_96\_RIGHT | TtGGCTCTGTTGGTGGGAATGT |

1 Variant position is shown against a SARS-CoV-2 reference genome 0MN908947.3.

2 Shown only prevalence >1%

3 Red lower case indicates mutation in the primer binding site.

**Table S4. SARS-CoV-2 clade comparison between the high-throughput 2-step PCR workflow and a standard ARTIC v3 workflow**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |  | |
|  |  | | **Clade by ARTIC v3 method** | | | | | | | | | | | | | |  | |  | |  | |
|  | Clade1 | 19B | | 20A | 20B | 20C | 20G | 20H (Beta, V2) | 20I (Alpha, V1) | 20J (Gamma, V3) | 21A (Delta) | 21C (Epsilon) | 21D (Eta) | 21F (Iota) | 21G (Lambda) | 21H | | Total | |  | |
| **Clade by 2-step PCR method** | 19B | | 3 |  |  |  |  |  |  |  |  |  |  |  |  |  | | 3 | |  | |
| 20A | |  | 53 |  |  |  |  |  |  |  |  |  |  |  |  | | 53 | |  | |
| 20B | |  |  | 105 |  |  |  | 1 |  |  |  |  |  |  |  | | 106 | |  | |
| 20C | |  |  |  | 98 |  |  |  |  |  |  |  |  |  |  | | 98 | |  | |
| 20G | |  |  |  |  | 175 |  |  |  |  |  |  |  |  |  | | 175 | |  | |
| 20H (Beta, V2) | |  |  |  |  |  | 7 |  |  |  |  |  |  |  |  | | 7 | |  | |
| 20I (Alpha, V1) | |  |  |  |  |  |  | 713 |  |  |  |  |  |  |  | | 713 | |  | |
| 20J (Gamma, V3) | |  |  |  |  |  |  |  | 44 |  |  |  |  |  |  | | 44 | |  | |
| 21A (Delta) | |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  | |  | |  | |
| 21C (Epsilon) | |  |  |  |  |  |  |  |  |  | 101 |  |  |  |  | | 101 | |  | |
| 21D (Eta) | |  |  |  |  |  |  |  |  |  |  | 10 |  |  |  | | 10 | |  | |
| 21F (Iota) | |  |  |  |  |  |  |  |  |  |  |  | 131 |  |  | | 131 | |  | |
| 21G (Lambda) | |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  | | 1 | |  | |
|  | 21H | |  |  |  |  |  |  |  |  |  |  |  |  |  | 3 | | 3 | |  | |
|  | Total | | 3 | 53 | 105 | 98 | 175 | 7 | 714 | 44 |  | 101 | 10 | 131 | 1 | 3 | | 1446 | |  | |
|  |  | |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |  | |

1 SARS-CoV-2 clades were assigned with Nextclade (<https://clades.nextstrain.org/>).

**Table S5. SARS-CoV-2 lineage comparison between the high-throughput 2-step PCR workflow and a standard ARTIC v3 workflow**

Chart

Description automatically generated

**Table S6. SARS-CoV-2 primer binding site mutations causing amplicon dropout by a standard ARTIC v3 workflow**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Clade** | **Number  Sample** | **Failed  Amplicon** | **Affected Primer** | **Primer Sequence**1 **(5' to 3')** | **Position**2 | **Reference** | **Variant** |
| 20H (Beta, V2) | 7 | ARTICv3-74 | nCoV-2019\_74\_LEFT | ACATCACTAGGTTTCAAAC(tttacttgc) | 22281 | C | -TTTACTTGC |
| 21A (Delta) | 1 | ARTICv3-72 | nCoV-2019\_72\_RIGHT | ACTC(tgaact)CACTTTCCATCCAAC | 22028 | G | -AGTTCA |
| 21C (Epsilon) | 113 | ARTICv3-72 | nCoV-2019\_72\_RIGHT | ACTCTGAACTCACTTTCCATaCAAC | 22018 | G | T |
| 21G (Lambda) | 1 | ARTICv3-97 | nCoV-2019\_97\_LEFT | TGGATGACAAAGATCCAAATTTtAAAGA | 29311 | C | T |
| nCoV-2019\_97\_RIGHT | ACACACTGATTAAAGATTaCTATGTGAG | 29675 | C | T |

1 Red lower case indicates mutation in the primer binding site; deleted nucleotides are shown in parentheses.

2 Variant position is shown against a SARS-CoV-2 reference genome 0MN908947.3.

**Table S7. Proportion of samples generating complete SARS-CoV-2 consensus sequence by the high-throughput workflow and a standard ARTIC v3 workflow**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **High-throughput workflow** | | | |  | **ARTIC v3 workflow** | | | |
| Clade | Number complete | Number incomplete | Total assessed | Percent complete |  | Number complete | Number incomplete | Total assessed | Percent complete |
| 20H (Beta, V2) | 4 | 1 | 5 | 80.0 |  | 16 | 69 | 85 | 18.8 |
| 21A (Delta) | 855 | 42 | 897 | 95.3 |  | 4 | 515 | 519 | 0.8 |
| 21C (Epsilon) | 7 | 0 | 7 | 100.0 |  | 5 | 256 | 261 | 1.9 |
| 21G (Lambda) | 15 | 3 | 18 | 83.3 |  | 1 | 29 | 30 | 3.3 |
| Total | 881 | 46 | 927 | 95.0 |  | 26 | 869 | 895 | 2.9 |

Different sample set was used between the two workflows.

Percent genome coverage was determined by counting the number of nucleotides meeting minimum coverage requirement divided by the total SARS-CoV-2 genome length excluding the 5’ (1-54nt) and 3’ (29,836–29,903nt) ends not covered by the amplicon panel.