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Dynamics of SARS-Cov-2 ORF7a Gene Deletions and Fate of Downstream ORF7b and ORF8 Genes Expression

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Abstract

SARS-CoV-2 small transactivator proteins like ORF7a, ORF7b and ORF8 were implicated as modulator of cellular genes like STAT1/2, Tom70, IRF3 to down regulate IFNγ, IL6 and IgG production. Genetic locus of those genes was frequently deleted and mutated with no expression of those small proteins and virus was cleared by host immune response more easily. We searched the ORF7a and ORF7b mutants in the NCBI database to characterize the genes and their presence in different variant and sub-variants were addressed to check if extinction of such mutated corona viruses with time. The ORF7a deletions were reported first in 2020 in early corona virus lineages but no further study was made. Thus, we reviewed the occurrence of such ORF7a gene deletions in alpha, delta and omicron corona viruses. Specific oligonucleotide at the deletion boundary was used to BLAST-N search for identical genes in the Database. Multi-alignment found ORF7a 60-63nt deletions in the carboxy-terminus which sometime also deleted the TAG termination codon of ORF7a as well as ATG initiation codon of ORF7b gene with no production of both functional proteins. In some situation a chimera ORF7a protein may be formed due to inactivation of TGA codon. More surprisingly, ORF7a/b deletion mutants also associated with creation of downstream TAA termination codon in the ORF8 gene which was shown as potential hotspot of at least one dozen mutations. We also detected two omicron BA.4 variant with three deletions in the ORF7a gene that also created two early termination codons. Such inactivation mechanisms were found dominant in omicron, alpha and few delta corona virus variants. The hairpin structures of ORF7a gene locus were changed (hairpin 6) in more than one deletion mutant and stiff hairpin 2 structure at the NH2 terminus might driver such deletion process. The associated important changes in the ORF1ab and spike proteins were also analysed to pinpoint variant status. Absence of expression of ORF7a, ORF7b and ORF8 proteins likely enhance SARS-CoV-2 clearance in host and may be one of the mechanisms of lower pathogenicity with time.

Keywords: SARS-CoV-2 deletions; ORF7a/b; ORF8 TAA termination mutant; Virus clearance; Low pathogenicity; Omicron corona virus

Introduction

SARS-CoV-2 is a large positive-stranded RNA virus with~30000 nucleotides genome. It has structural proteins Membrane (M), Envelope (E), Nucleocapsid (N), Spike (S) coded from 3'-1/3 part of the virus independently but RNA-dependent RNA polymerase was coded from nsp12 domain of ORF1ab polyprotein coded from 2/3 of the 5'-part of the genome [1]. ORF1ab polyprotein was degraded into sixteen polypeptides (nsp1-nsp16) (Figure 1). The nsp2 protein is RNA topoisomerase whereas Nsp3 and nsp5 are proteases [2]. The nsp6, nsp7, nsp8, nsp9 and nsp10 were small accessory proteins involved in RNA polymerase replication complex. The nsp14 and nsp15 are nucleases and nsp16 is methyltransferase and as well as nsp13 is RNA helicase [3-5]. Nsp11 is a small peptide and function was not known. The ORF3a, ORF6, ORF7a/b, ORF8, ORF9 and ORF10 small proteins also coded from 3' end of the SARS-CoV-2 genome and have roles in regulating cellular genes [6]. Many drugs were discovered against proteases and RNA polymerases but vaccines were only important remedy that halted the corona virus spread [7]. The most frequent mutation that occurred in most corona virus isolates was 3037C>T which is a synonymous change that usually accompanied 3 other mutations that include 241C>T, 14408C>T (P323L in RdRp) and 23403A>G (D614G in S-protein). ORF7a gene is a hotspot for deletion mutations but mechanism of such changes was poorly understood [8]. More 29 mutations in spike of omicron corona viruses with concurrent D614G and N501Y mutations caused antibody escape with mild diseases. Those mutations were: A67V (V67), T95I (I93), N211I (I206), L212V (V207), V215P (P210), R216E (E211), G341D (D336), S373L (L368), S375P (P370), S377F (F372), K419N (N414), N442K (K437), G448S (S443), S479N (N474), E486A (A481), Q495R (R490), G498S (S493), Q500R (R495), Y507H (H502), T549K (K544), H657Y (Y652), P683H (H678), N766K (K761), D798Y (Y793), N858K (K853), Q956H (H951), N971K (K966), and L983F (F978) [in sate values for omicron virus positions] [9]. The ORF7a is a type-I transmembrane protein of 121 amino acid residues with an N-terminal signal peptide (residues 1–15), an immunoglobulin (Ig)-like ectodomain (residues 16–96), a transmembrane domain (residues 97–116), and an ER retention motif (residues 117–121) (see, figure-14). About 872nt deletion in the ORF7a/b locus was reported expressing stable corona virus isolates [10,11]. Sequencing of SARS-CoV-2 led to discovery of 382nt deletion in the ORF7a/b-ORF8 genes (see, accession no. MT374101) [12-14]. Further study indicated two medium (81nt and 96nt) deletion mutants at the NH2 terminus of ORF7a and two other medium deletion (40nt and 47nt) in the carboxy terminus of ORF7a gene [15-17]. ORF7a subverts phosphorylation of STAT2, suppressing transcriptional activation of antiviral IFN-stimulated genes that can otherwise restrict viral replication [18]. Although the proposed function for ORF7a is intracellular, antibodies against ORF7a are elevated in the serum of COVID-19 patients. Preliminary studies indicated that recombinant ORF7a protein interacts with CD14+ monocytes and triggers expression of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-a). However, it was unclear if those mutants interacted badly in COVID-19 patients. We extended such studies and reviewed in details how similar ORF7a gene deletions (~63nt) were incorporated in recent corona virus variants. In Vero cell culture ORF7a defective viruses grew slow indicating ORF7a protein was necessary for viral replication [19]. We deeply characterized the different ORF7a deletion mutants to demonstrate their spread into alpha, delta and omicron corona virus variants.

Methods

We searched PubMed to get idea on published papers on ORF8 and also searched SARS-CoV-2 NCBI database using BLAST-N and BLAST-X search methods. Multi-alignment of protein was done by MultAlin software and multi-alignment of DNA by CLUSTAL-Omega software, EMBL-EBI [20]. The ORF8 mutants was obtained by BlastN search of deletion boundary of ~120nt sequence and then analyzing the sequences with 90-100% similarities. Blast-X search of ORF7a deleted gene used to get more deletion mutants. Then, the other ORF7a mutants were detected by Blast-N search with selected deletion boundaries. Hairpin structure of ORF7a gene~ 200nt sequence was done by OligoAnalyzer 3.1 software (Integrated DNA Technologies). The protein 3-D structure was determined by SWISS-Model software [21].

Result

We got the idea of ORF7a deletion (accession no. OP711842) from our earlier work on ORF8 inactivation with creation of TAA termination mutation (Figure 2). BlastN search with deletion boundary gave ten sequences with 100% homology and 100% cover (Figure 3). The oligonucleotides of few sequences at the deletion boundaries were given in Table 1. The sequences with accession nos. MW309830, MW309829 and MW309831 were obtained from earlier studies in 2020 whereas we found here only one sequences belonged to omicron variant (Table 1). Table 1 showed that only ORF7a deletion mutants with accession numbers OK249834, OL447329, MT628273, ON926042 and MW309829 were spread into omicron variants. The figure 4 showed the Blast-2 search between oligonucleotides with mutant genomes to confirm the authenticity of oligonucleotides (Figure 4). Few ORF7a deletion abolished ATG codon of ORF7b gene (accession numbers OL522556, OK249824, OK192887, MZ727798, OP334687, OW352845) while in few ORF7a mutants ATG codon of ORF7b was retained (accession numbers OP711837, OL447329, OU556481, OP398207, OK439931, ON926042) (Figure 5). In (Figure 6), we demonstrated that few ORF7a deletion mutants had consecutive three deletions (accession nos. OP174404 and OP490708; Omicron BA.4 variant). The first deletion was 85nt at 27471 (5'-aca aca gta ctt tta aaa gaa cct tgc tct tct gga aca tac gag ggc aat tca cca ttt cat cct cta gct gat aac aaa ttt g-3'), followed by second 45nt deletion at 27608 (5'-aca cgt cta tca gtt acg tgc cag atc agt ttc acc taa act gtt-3') and a third deletion of 63nt at 27687 (5'-cca att ttt ctt att gtt gcg gca ata gtg ttt ata aca ctt tgc ttc aca ctc aaa aga aag-3') but no change in ORF7b and ORF8 genes. Such truncation also created TGA and TAA two termination codons but nullifying its own TGA termination codon facilitating no ORF7a protein synthesis (Figure 6). We demonstrated the BlastX data of ORF7a/b-ORF8 locus of two mutants. In mutant -1 (accession no. ON940286) ORF7a gene fusion protein may be obtained but ORF7b and ORF8 protein could made normal (Table 2). Whereas in mutant-2 (accession no. OK249834), no ORF7a and also ORF7b due to deletion of ATG codon as well as ORF8 due to creation of TAA termination codon. Thus, we clearly demonstrated the hotspot

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deletion site of the ORF7a/b-ORF8 locus. In figure-7, we presented few ORF7a 96nt deletion mutants belong to omicron BA.2 and BA.4 corona viruses as well as B.1.1.7 lineage (Table 3) (Figure 7). We made primers to amplify such deletion sequences and made restriction analysis to characterize the mutant sequences (Figure 9). We showed the multi-alignment of spike protein of ORF7a deletion mutants to demonstrate their variant status. The OM966459 sequence was omicron BA.1 as it had 143VYY deletion and 215EPE insertion in the spike. The MT628273, ON577132, ON577132 sequences were early lineages as no ~30 mutations in the RBD domain of spike.

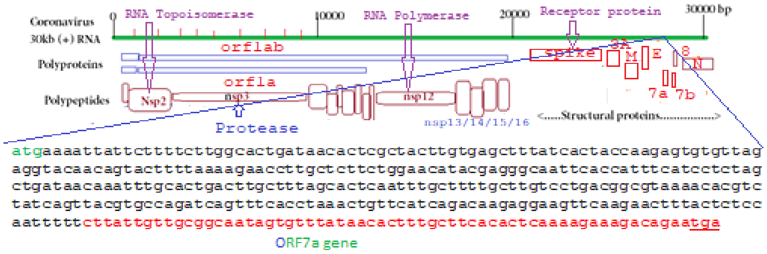

Figure 1: *Genetic structure of SARS-CoV-2 and complete sequence of the ORF7a gene. Red colour indicated frequent deletion in this region of the ORF7a gene. The ORF7a termination codon and ORF7b initiation codon were merged.*

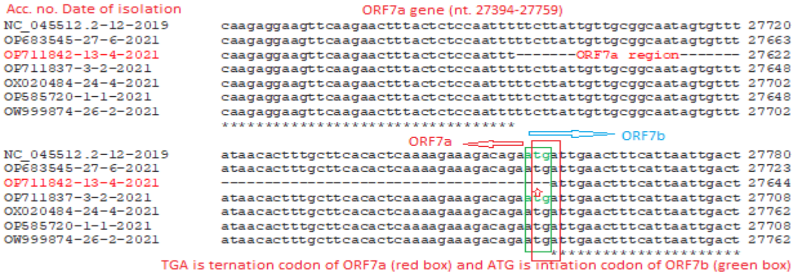

Figure 2: *Detection of 3'-end of the ORF7a gene following inactivation of ORF7b gene in ORF8 gene (not shown here) TAA termination codon mutants of corona virus.*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/KEN/KEM-21-04-99437/2021 O...	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29631	OP711842.1
☑	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NC-CDC-LC0842822/2022...	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29644	OP334687.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW962857.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW376523.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW353845.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, complete genome: monopartite	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW414598.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW351812.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW266488.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW265376.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW236085.1
☑	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	82.4	82.4	100%	2e-12	100.00%	29771	OW141269.1

Figure 3: *BLAST-N search with deletion boundary oligo to get ORF7a deletion mutants. Only 10 mutants were found with 100% homology with 100% cover.*

Table 1: *Deletion boundary oligonucleotides used to trap ORF7a deleted mutants in the database.*

Acc. No.	Oligo sequence	No.	Variant
OP711842	5'-caagaactttactctccaatttattgaactttcattaattgact-3' (44nt)	10	B.1.1.7
OK249834	5'-caagaactttactctccaattttattgaactttcattaattgact-3'(45nt)	27	B.1.1.7
OD995649	5'-caagaactttactctccaatttttcttgaactttcattaattgact-3'(46nt)	3	B.1.1.7
OV692136	5'-caagaactttactctccaatttttattgaactttcattaattgact-3'(46nt)	4	BA.1
OK439931	5'-caagaactttactctccaatttttaatgattgaactttcattaattgact-3'(50nt)	6	B.1
OL447329	5'-caagaactttactctccaattgaatgattgaactttcattaattgact-3'(48nt)	33	B.1.617.2
OU911410	5'-caagaactttactctccaattttgaatgattgaactttcattaattgact-3'(51nt)	3	B.1.617.2

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MT628273	5’-caagaactttactctacagaatgattgaactttcattaattgact-3’(45nt)	40	BA.2
ON926042	5’-caagaactttactctccaatttttacagaatgattgaactttcattaattg-3’(51nt)	54	BA.2
MW309830	5’-tttgcactgacttgctttagcactcaagtttcacctaaactgttcatcag-3’(50nt)	2	A.3
MW309829	5’-caccatttcatcctctagctgataacaaactgttcatcagacaagagg-3’(48nt)	34	B.1
MW309831	5’-aactttactctccaatttttcttataaaagaaagacagaatgattgaac-3’(49nt)	6	B.1.36
OP490708 (three deletions)	5’atgaaaattattcttttcttggcactgataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtcactgacttgctttagcactcaatttgcttttgcttgtcctgacggcgtaaacatcagacaagaggaagttcaagaactttactctacagaatga3’	2	BA.4

```
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NC-CDC-LC0842822/2022.
Sequence ID: OP334687.1Length: 29644. (same as OP711842) .  (Score=10)
Query  1      CAAGAACTTTACTCTCCAATTTATTGAACTTTCATTAATTGACT  44
              ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27598  CAAGAACTTTACTCTCCAATTTATTGAACTTTCATTAATTGACT  27641
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1/Sequence
ID: OV692136.1Length: 29808.(same as OV692136) .  (score=4)
Query  1      CAAGAACTTTACTCTCCAATTTTTATTGAACTTTCATTAATTGACT  46
              ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27649  CAAGAACTTTACTCTCCAATTTTTATTGAACTTTCATTAATTGACT  27694
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/PA-CDC-STM-000024188/2021.
Sequence ID: OK440204.1Length: 29814.(same as OK439931) . (score=6)
Query  1      CAAGAACTTTACTCTCCAATTTTTAATGATTGAACTTTCATTAATTGACT  50
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27643  CAAGAACTTTACTCTCCAATTTTTAATGATTGAACTTTCATTAATTGACT  27692
Severe acute respiratory syndrome coronavirus 2 isolate hCoV-19/Switzerland/NE-ETHZ-370111/2020 genome,
Sequence ID: OE997978.1Length: 29774. (Same as OD995649) . (score=3)
Query  1      CAAGAACTTTACTCTCCAATTTTTCTTGAACTTTCATTAATTGAC  45
              |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27643  CAAGAACTTTACTCTCCAATTTTTCTTGAACTTTCATTAATTGAC  27687
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1
Sequence ID: OU911410.1Length: 29824. (same as OU911410) .  (score=3)
Query  1      CAAGAACTTTACTCTCCAATTTTTGAATGATTGAACTTTCATTAATTGACT  51
              |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27658  CAAGAACTTTACTCTCCAATTTTTGAATGATTGAACTTTCATTAATTGACT  27708
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/MEX/ROO-5_2141/2021.
Sequence ID: ON487207.1Length: 29685. (same as OL447329) (score=27)
Query  1      CAAGAACTTTACTCTCCAATTGAATGATTGAACTTTCATTAATTGACT  48
              ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27605  CAAGAACTTTACTCTCCAATTGAATGATTGAACTTTCATTAATTGACT  27652
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/MEX/ROO-5_2141/2021.
Sequence ID: ON487207.1Length: 29685. (Same as  OL447329) .  (score=33)
Query  1      CAAGAACTTTACTCTCCAATTGAATGATTGAACTTTCATTAATTGACT  48
              ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27605  CAAGAACTTTACTCTCCAATTGAATGATTGAACTTTCATTAATTGACT  27652
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CZB-1621/2020,
Sequence ID: MT628273.1Length: 29752. (same as ON940286) .  (Score=40)
Query  1      CAAGAACTTTACTCTACAGAATGATTGAACTTTCATTAATTGACT  45
              |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27635  CAAGAACTTTACTCTACAGAATGATTGAACTTTCATTAATTGACT  27679
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NY-CDC-LC0917472/2022.
Sequence ID: OP753941.1Length: 29603. (Same as ON926042) . (score=54)
Query  1      CAAGAACTTTACTCTCCAATTTTTACAGAATGATTGAACTTTCATTAATTGACT  54
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27547  CAAGAACTTTACTCTCCAATTTTTACAGAATGATTGAACTTTCATTAATTGACT  27600
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Figure 4: *BLAST-N search to demonstrate the authenticity of ORF7a deletion boundary oligonucleotides.*

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OL522556-22-6-2021       ttcatcagacaagaggaagttcaagaactttactctccaatttt------F---------27677
OK249834-20-6-2021       ttcatcagacaagaggaagttcaagaactttactctccaatttt------F---------27623
OK192887-20-4-2021       ttcatcagacaagaggaagttcaagaactttactctccaatttt------F---------27647
MZ727798-28-6-2021       ttcatcagacaagaggaagttcaagaactttactctccaatttt------F---------27623
OD995649-15-2-2021       ttcatcagacaagaggaagttcaagaactttactctccaattttt-----F---------27678
OP711842-13-4-2021       ttcatcagacaagaggaagttcaagaactttactctccaattt-------T---------27622
OP711837-3-2-2021        ttcatcagacaagaggaagttcaagaactttactctccaatttttcttattgttgcggca27639
OK439931-18-2-2021       ttcatcagacaagaggaagttcaagaactttactctccaattttt-----F---------27694
B-NC_045512.2-12-2019    ttcatcagacaagaggaagttcaagaactttactctccaatttttcttattgttgcggca27711
OE997012-29-12-2020      ttcatcagacaagaggaagttcaagaactttactctccaatttt------F---------27692
OL447329-12-10-2021      ttcatcagacaagaggaagttcaagaactttactctccaattg-------T---------27646
OU556481-10-8-2021       ttcatcagacaagaggaagttcaagaactttactctccaattg-------T---------27688
OP398207-3-8-2021        ttcatcagacaagaggaagttcaagaactttactctccacag--------F---------27666
OM766944-3-2-2022        ttcatcagacaagaggaagttcaagaactttactctccaatttt------F---------27634
OP334687-17-8-2022       ttcatcagacaagaggaagttcaagaactttactctccaattt-------T---------27619
OW353845-19-3-2022       ttcatcagacaagaggaagttcaagaactttactctccaattt-------T---------27661
OW265376-14-3-2022       ttcatcagacaagaggaagttcaagaactttactctccaattt-------T---------27661
ON940286-20-6-2022       ttcatcagacaagaggaagttcaagaactttactc---------------F---------27575
ON926042-4-5-2022        ttcatcagacaagaggaagttcaagaactttactctccaatttt------T---------27683
                         ***********************************

OL522556-22-6-2021       -----------------------------------------------attgaactttcat27690
OK249834-20-6-2021       -----------------------------------------------attgaactttcat27636
OK192887-20-4-2021       -----------------------------------------------attgaactttcat27660
MZ727798-28-6-2021       -----------------------------------------------attgaactttcat27636
OD995649-15-2-2021       -----------------------------------------------cttgaactttcat27691
OP711842-13-4-2021       -----------------------------------------------attgaactttcat27635
OP711837-3-2-2021        atagtgtttataacactttgcttcacactcaaaagaaagacagaatgattgaactttcat27699
OK439931-18-2-2021       -------------------------------------------aatgattgaactttcat27711
B-NC_045512.2-12-2019    atagtgtttataacactttgcttcacactcaaaagaaagacagaatgattgaactttcat27771
OE997012-29-12-2020      -----------------------------------------------attgaactttcat27705
OL447329-12-10-2021      -------------------------------------------aatgattgaactttcat27663
OU556481-10-8-2021       -------------------------------------------aatgattgaactttcat27705
OP398207-3-8-2021        -------------------------------------------aatgattgaactttcat27683
OM766944-3-2-2022        -----------------------------------------------attgaactttcat27647
OP334687-17-8-2022       -----------------------------------------------attgaactttcat27632
OW353845-19-3-2022       -----------------------------------------------attgaactttcat27674
OW265376-14-3-2022       -----------------------------------------------attgaactttcat27674
ON940286-20-6-2022       --------------------------------------tacagaatgattgaactttcat27588
ON926042-4-5-2022        --------------------------------------tacagaatgattgaactttcat27696
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Figure 5: *Multi-alignment of ORF7a deletion mutant genomes as compared to NC_045512.2. “T” means normal termination of ORF7a deletion mutants and “F” means fusion ORF7a protein may be produced. Part of the alignment was shown here.*

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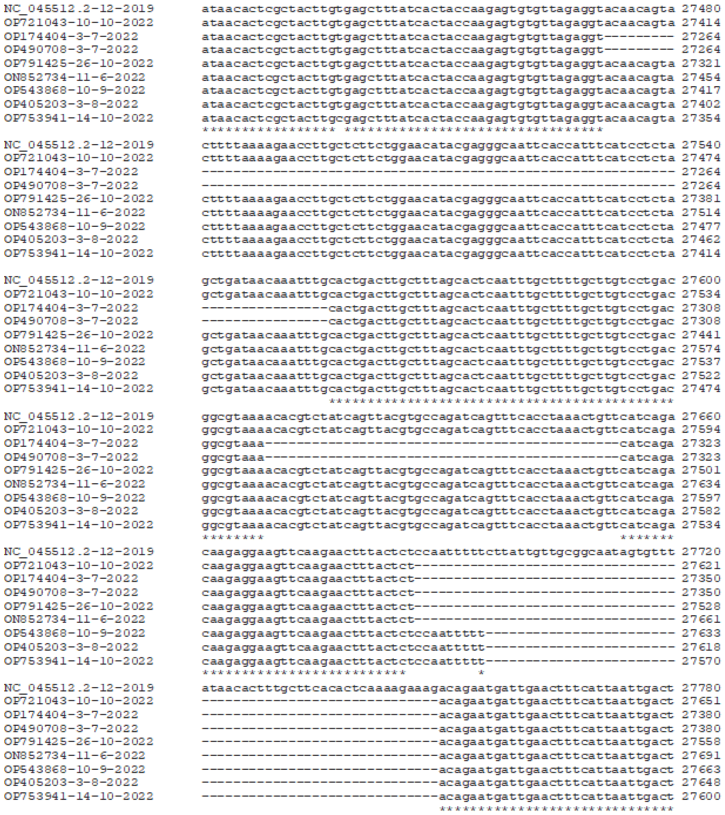
Table 2: *Fusion protein or termination of ORF7a protein due to extended ORF7b and ORF8 genes and status of ORF7b and ORF8 protein in ORF7a deletion mutants.*

ON940286	5’-caattagatgaagagcaaccaatggagattctctaaacgaac**atg**aaaattattcttttcttggcactgataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtacaacagtacttttaaaagaaccttgctcttctggaacatacgagggcaattcaccatttcatcctctagctgataacaaatttgcactgacttgctttagcactcaatttgcttttgcttgtcctgacggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgcaagaactttactctacaga**atg**attgaactttcattaattgacttctatttgtgctttttagcctttctgttattccttgttttaattatgcttattatcttttggttctcacttgaactgcaagatcataatgaaacttgtcacgcctaaacgaac**atg**aaatttcttgttttcttaggaatcatcacaactgtagctgcatttcaccaagaatgtagtttacagtcatgtactcaacatcaaccatatgtagttgatgacccgtgtcctattcacttctattctaaatggtatattagagtaggagctagaaaatcagcacctttaattgaattgtgcgtggatgaggctggttctaaatcacccattcagtacatcgatatcggtaattatacagtttcctgtttaccttttacaattaattgccaggaacctaaattgggtagtcttgtagtgcgttgttcgttctatgaagactttttagagtatcatgacgttcgtgttgttttagatttcatctaaacgaacaaacttaaatgtctgataatggaccccaaaatcagcgaaatgcact-3’	F
blastX	**ORF7a protein [SARS-CoV-2]Sequence ID: UWV44657.1Length: 121 AA.** Query 8 MKiilflalitlatCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFS 187 MKIILF ALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFS Sbjct 1 MKIILFFALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFS 60 Query 188 TQFAFACPDGVKHVYQLRARSVSPKL-------QELYST 283 TQFAFACPDGVKHVYQLRARSVSPKL QELYS+ Sbjct 61 TQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSS 99 **ORF7b [SARS-CoV-2]Sequence ID: UOB38331.1Length: 43 AA.** Query 19 MIELSLIDfylcflafllflvlimliifwfSLELQDHNETCHA 147 MIELSLIDFYLCFLAFLLF+VLIMLIIFWFSLELQDHNETCHA Sbjct 1 MIELSLIDFYLCFLAFLLFVVLIMLIIFWFSLELQDHNETCHA 43 **ORF8 protein [SARS-CoV-2]Sequence ID: YP_009724396.1Length: 121 AA.** Query 459 MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGARKSAPLIEL 638 MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGARKSAPLIEL Sbjct 1 MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGARKSAPLIEL 60 Query 639 CVDEAGSKSPIQYIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDF I 821 CVDEAGSKSPIQYIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDF Sbjct 61 CVDEAGSKSPIQYIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDF I 121	
OK249834	5’-Caattagatgaagagcaaccaatggagattgattaaacgaac**atg**aaaattattcttttcttggcactgataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtacaacagtacttttaaaagaaccttgctcttctggaacatacgagggcaattcaccatttcatcctctagctgataacaaatttgcactgacttgctttagcacttaatttgcttttgcttgtcctgacggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgcaagaactttactctccaattttattgaactttcattaattgacttctatttgtgctttttagcctttctgctattccttgttttaattatgcttattatcttttggttctcacttgaactgcaagatcataatgaaacttgtcacgcctaaacgaac**atg**aaatttcttgttttcttaggaatcatcacaactgtagctgcatttcaccaagaatgtagtttacagtcatgtacttaacatcaaccatatgtagttgatgacccgtgtcctattcacttctattctaaatggtatattagagtaggagctataaaatcagcacctttaattgaattgtgcgtggatgaggctggttctaaatcacccattcagtgcatcgatatcggtaattatacagtttcctgtttaccttttacaattaattgccaggaacctaaattgggtagtcttgtagtgcgttgttcgttctatgaagactttttagagtatcatgacgttcgtgttgttttagatttcatctaaacgaacaaactaaatgtctctaaatggaccccaaaatcagcgaaatgcacc-3’	T
blastX	**ORF7a protein [SARS-CoV-2]Sequence ID: URK29908.1Length: 121 AA.** Query 7 MKiilflalitlatCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFS 186 MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFS Sbjct 1 MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFS 60 Query 187 T*FAFACPDGVKHVYQLRARSVSPKL-------QELYSPILL 291 T FAFACPDGVKHVYQLRARSVSPKL QELYSPILL Sbjct 61 TQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPILL 102 **ORF8 protein [SARS-CoV-2]Sequence ID: QRX60686.1Length: 121.** Query 459 MKFLVFLGIITTVAAFHQECSLQSCT*HQPYVVDDPCPIHFYSKWYIRVGAIKSAPLIEL 638 MKFLVFLGIITTVAAFHQECSLQSCT HQPYVVDDPCPIHFYSKWYIRVGAIKSAPLIEL Sbjct 1 MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGAIKSAPLIEL 60 Query 639 CVDEAGSKSPIQCIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDF I 821 CVDEAGSKSPIQCIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDF Sbjct 61 CVDEAGSKSPIQCIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDF I 121	

Table 3: *Primer sequence for PCR of 419/423bp ORF7a deletion boundary (normal length=504bp).*

Forward	5’-GCTTTTGCTTGTCCTGACGG-3’	Tm=60.04^{0}C	GC=55%
Reverse	5’-GCCTCATCCACGCACAATTC-3’	Tm=59.9^{0}C	GC=55%

Citation: Chakraborty AK (2022). Dynamics of SARS-Cov-2 ORF7a Gene Deletions and Fate of Downstream ORF7b and ORF8 Genes Expression. SunText Rev Biotechnol 3(1): 142.



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OP174404-3-7-2022       ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggt--------- 27264
OP490708-3-7-2022       ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggt--------- 27264
OP791425-26-10-2022     ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtacaacagta 27321
ON852734-11-6-2022      ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtacaacagta 27454
OP543868-10-9-2022      ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtacaacagta 27417
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OP753941-14-10-2022     ataacactcgctacttgcgagctttatcactaccaagagtgtgttagaggtacaacagta 27354
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OP174404-3-7-2022       ------------------------------------------------------------ 27264
OP490708-3-7-2022       ------------------------------------------------------------ 27264
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OP753941-14-10-2022     cttttaaaagaaccttgctcttctggaacatacgagggcaattcaccatttcatcctcta 27414

NC_045512.2-12-2019     gctgataacaaatttgcactgacttgctttagcactcaatttgcttttgcttgtcctgac 27600
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OP174404-3-7-2022       ----------------cactgacttgctttagcactcaatttgcttttgcttgtcctgac 27308
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ON852734-11-6-2022      gctgataacaaatttgcactgacttgctttagcactcaatttgcttttgcttgtcctgac 27574
OP543868-10-9-2022      gctgataacaaatttgcactgacttgctttagcactcaatttgcttttgcttgtcctgac 27537
OP405203-3-8-2022       gctgataacaaatttgcactgacttgctttagcactcaatttgcttttgcttgtcctgac 27522
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                                        ********************************************

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OP174404-3-7-2022       ggcgtaaa---------------------------------------------catcaga 27323
OP490708-3-7-2022       ggcgtaaa---------------------------------------------catcaga 27323
OP791425-26-10-2022     ggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgttcatcaga 27501
ON852734-11-6-2022      ggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgttcatcaga 27634
OP543868-10-9-2022      ggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgttcatcaga 27597
OP405203-3-8-2022       ggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgttcatcaga 27582
OP753941-14-10-2022     ggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgttcatcaga 27534
                        ********                                             *******
NC_045512.2-12-2019     caagaggaagttcaagaactttactctccaatttttcttattgttgcggcaatagtgttt 27720
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OP490708-3-7-2022       caagaggaagttcaagaactttactct--------------------------------- 27350
OP791425-26-10-2022     caagaggaagttcaagaactttactct--------------------------------- 27528
ON852734-11-6-2022      caagaggaagttcaagaactttactct--------------------------------- 27661
OP543868-10-9-2022      caagaggaagttcaagaactttactctccaatttt------------------------- 27633
OP405203-3-8-2022       caagaggaagttcaagaactttactctccaatttt------------------------- 27618
OP753941-14-10-2022     caagaggaagttcaagaactttactctccaatttt------------------------- 27570
                        ***************************       *

NC_045512.2-12-2019     ataacactttgcttcacactcaaaagaaagacagaatgattgaactttcattaattgact 27780
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OP490708-3-7-2022       ------------------------------acagaatgattgaactttcattaattgact 27380
OP791425-26-10-2022     ------------------------------acagaatgattgaactttcattaattgact 27558
ON852734-11-6-2022      ------------------------------acagaatgattgaactttcattaattgact 27691
OP543868-10-9-2022      ------------------------------acagaatgattgaactttcattaattgact 27663
OP405203-3-8-2022       ------------------------------acagaatgattgaactttcattaattgact 27648
OP753941-14-10-2022     ------------------------------acagaatgattgaactttcattaattgact 27600
                                                      ******************************
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Figure 6: *Demonstration of two and three deletions in the ORF7a gene as well as with other accompanying ~63nt single deletion mutants. ORF7a gene = nt. 27394-27759, ORF7b gene = nt. 27750-27887 and ORF8 gene = nt. 27894-28259 (accession no. NC_045512.2).*

```
Acc. no/Date of isolation             96nt ORF7a gene                                 Position/Variant
OP138059-16-7-2022      gctgataac---------------------------------------------------   27466 BA.4
OP258059-31-7-2022      gctgataac---------------------------------------------------   27466 BA.4
ON729188-28-5-2022      gctgataac---------------------------------------------------   27396 BA.2
ON801548-2-6-2022       gctgataac---------------------------------------------------   27492 BA.2
MZ780448-29-4-2021      gctgataac---------------------------------------------------   27525 B.1.1.7
NC_045512-12-2019       gctgataacaaatttgcactgacttgctttagcactcaatttgcttttgcttgtcctgac   27600 B
MW309829-24-3-2020      gctgataac---------------------------------------------------   27495 B.1
                        *********

OP138059-16-7-2022      ---------------------------------------------aaactgttcatcaga   27481 BA.4
OP258059-31-7-2022      ---------------------------------------------aaactgttcatcaga   27481 BA.4
ON729188-28-5-2022      ---------------------------------------------aaactgttcatcaga   27411 BA.2
ON801548-2-6-2022       ---------------------------------------------aaactgttcatcaga   27507 BA.2
MZ780448-29-4-2021      ---------------------------------------------aaactgttcatcaga   27540 B.1.1.7
NC_045512-12-2019       ggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgttcatcaga   27660 B
MW309829-24-3-2020      ---------------------------------------------aaactgttcatcaga   27510 B.1
                                                                     ***************
```

Figure 7: *The 96nt ORF7a gene deletion mutant (accession no. MW309829) discovered in 2020 in B.1 lineage. Now we found such deletion in B.1.1.7 lineage (2021) and Omicron BA.2 and BA.4 lineages (2022).*

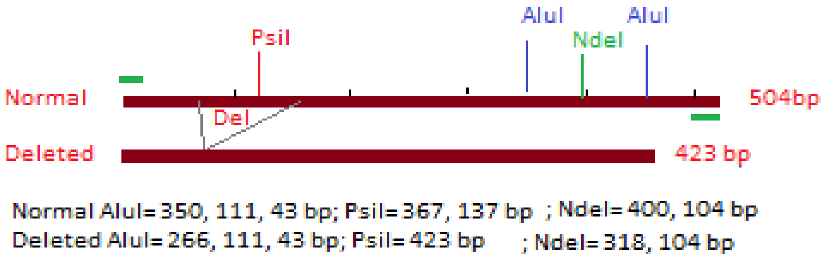
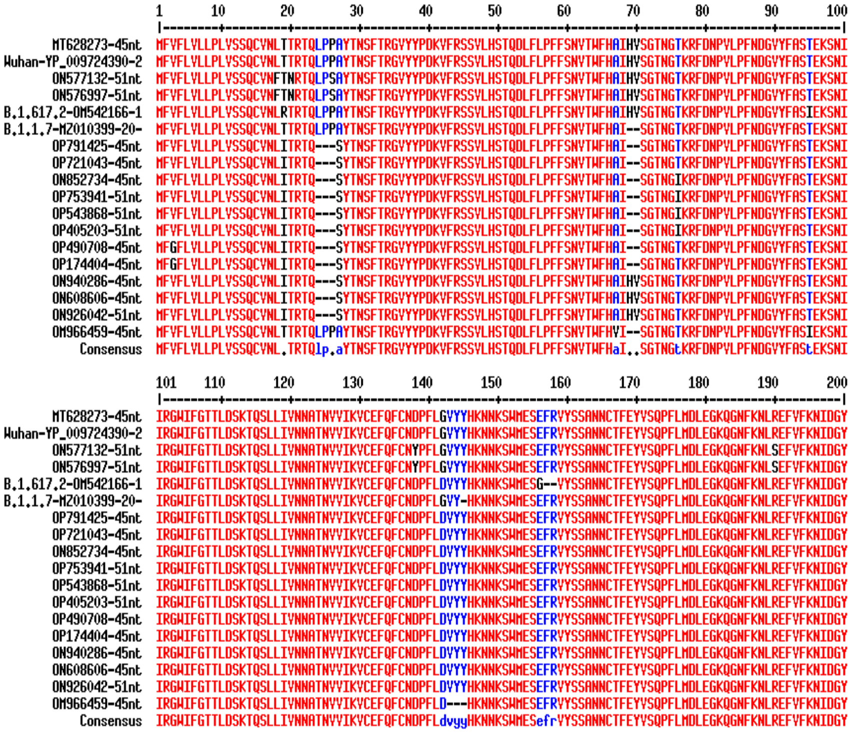


Figure 9: *Restriction analysis of ORF7a gene normal vs mutant PCR product.*



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                     1       10        20        30        40        50        60        70        80        90       100
                     |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
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Wuhan-YP_009724390-2 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI
       ON577132-51nt MFVFLVLLPLVSSQCVNFTNRTQLPSAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI
       ON576997-51nt MFVFLVLLPLVSSQCVNFTNRTQLPSAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI
B.1.617.2-OM542166-1 MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASIEKSNI
B.1.1.7-MZ010399-20- MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI--SGTNGTKRFDNPVLPFNDGVYFASTEKSNI
       OP791425-45nt MFVFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI--SGTNGTKRFDNPVLPFNDGVYFASTEKSNI
       OP721043-45nt MFVFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI--SGTNGTKRFDNPVLPFNDGVYFASTEKSNI
       ON852734-45nt MFVFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI--SGTNGIKRFDNPVLPFNDGVYFASTEKSNI
       OP753941-51nt MFVFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI--SGTNGIKRFDNPVLPFNDGVYFASTEKSNI
       OP543868-51nt MFVFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI--SGTNGIKRFDNPVLPFNDGVYFASTEKSNI
       OP405203-51nt MFVFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI--SGTNGIKRFDNPVLPFNDGVYFASTEKSNI
       OP490708-45nt MFGFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI--SGTNGTKRFDNPVLPFNDGVYFASTEKSNI
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       ON940286-45nt MFVFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI
       ON608606-45nt MFVFLVLLPLVSSQCVNLITRTQ---SYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI
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       OM966459-45nt MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHVI--SGTNGTKRFDNPVLPFNDGVYFASIEKSNI
           Consensus MFVFLVLLPLVSSQCVNL.TRTQlp.aYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHaI..SGTNGtKRFDNPVLPFNDGVYFAStEKSNI

                     101    110       120       130       140       150       160       170       180       190       200
                     |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
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Wuhan-YP_009724390-2 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
       ON577132-51nt IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNYPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLSEFVFKNIDGY
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B.1.617.2-OM542166-1 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLDVYYHKNNKSWMESG--VYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
B.1.1.7-MZ010399-20- IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVY-HKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
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       OP405203-51nt IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLDVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
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       OP174404-45nt IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLDVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
       ON940286-45nt IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLDVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
       ON608606-45nt IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLDVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
       ON926042-51nt IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLDVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
       OM966459-45nt IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLD---HKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
           Consensus IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLdvyyHKNNKSWMESefrVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
```
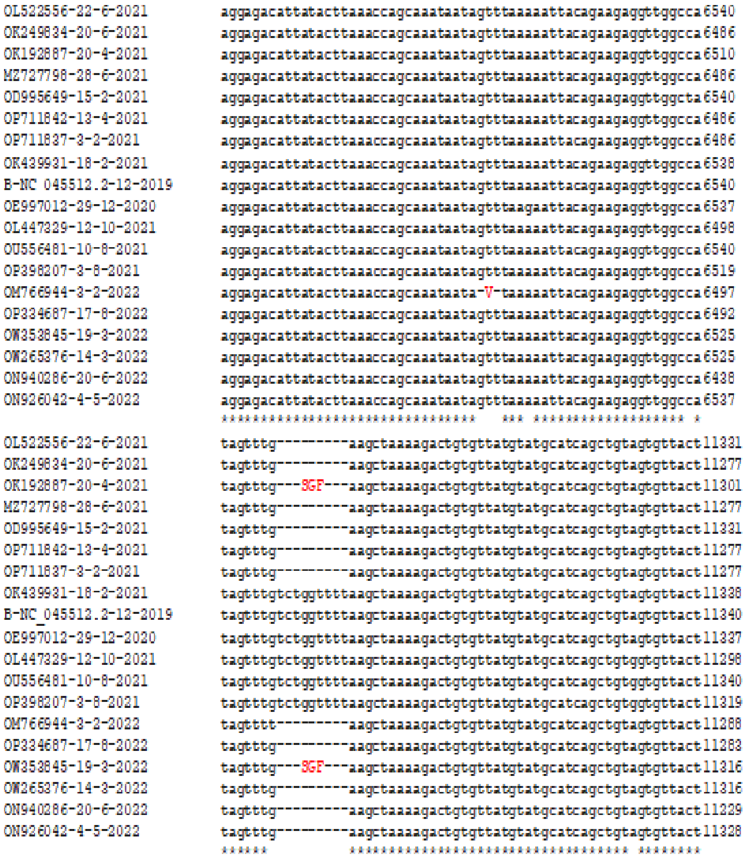

Figure 10: *Multi-alignment of Spike protein sequences selected by two deletion boundary oligos (45nt and 51nt) to demonstrate omicron corona virus with 24LPP and or 69HV deletions. Such isolates also have 31ERS deletion in N-protein (not shown here).*



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OP711837-3-2-2021        aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6486
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B-NC 045512.2-12-2019    aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6540
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OL447329-12-10-2021      aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6498
OU556481-10-8-2021       aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6540
OP398207-3-8-2021        aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6519
OM766944-3-2-2022        aggagacattatacttaaaccagcaaataata-V-taaaaattacagaagaggttggcca6497
OP334687-17-8-2022       aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6492
OW353845-19-3-2022       aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6525
OW265376-14-3-2022       aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6525
ON940286-20-6-2022       aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6438
ON926042-4-5-2022        aggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttggcca6537
                         ********************************   *** ******************* *

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MZ727798-28-6-2021       tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact11277
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OP711837-3-2-2021        tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact11277
OK439931-18-2-2021       tagtttgtctggttttaagctaaaagactgtgttatgtatgcatcagctgtagtgttact11338
B-NC_045512.2-12-2019    tagtttgtctggttttaagctaaaagactgtgttatgtatgcatcagctgtagtgttact11340
OE997012-29-12-2020      tagtttgtctggttttaagctaaaagactgtgttatgtatgcatcagctgtagtgttact11337
OL447329-12-10-2021      tagtttgtctggttttaagctaaaagactgtgttatgtatgcatcagctgtggtgttact11298
OU556481-10-8-2021       tagtttgtctggttttaagctaaaagactgtgttatgtatgcatcagctgtggtgttact11340
OP398207-3-8-2021        tagtttgtctggttttaagctaaaagactgtgttatgtatgcatcagctgtggtgttact11319
OM766944-3-2-2022        tagtttt---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact11288
OP334687-17-8-2022       tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact11283
OW353845-19-3-2022       tagtttg---SGF---aagctaaaagactgtgttatgtatgcatcagctgtagtgttact11316
OW265376-14-3-2022       tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact11316
ON940286-20-6-2022       tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact11229
ON926042-4-5-2022        tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact11328
                         ******          *********************************** ********
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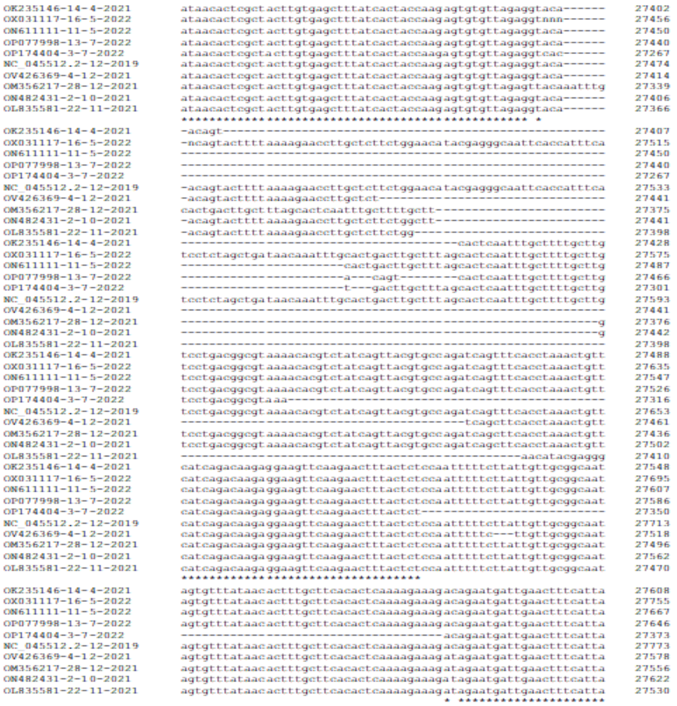
Figure 11: *Demonstration of ORF1ab gene deletions in few ORF7a deletion mutants. Only parts of the alignment shown where deletions were seen.*



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ON611111-11-5-2022         ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtaca------    27450
OP077998-13-7-2022         ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtaca------    27440
OP174404-3-7-2022          ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtcac------    27267
NC_045512.2-12-2019        ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtaca------    27474
OV426369-4-12-2021         ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtaca------    27414
OM356217-28-12-2021        ataacactcgctacttgtgagctttatcactaccaagagtgtgttagagttacaaatttg    27339
ON482431-2-10-2021         ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtaca------    27406
OL835581-22-11-2021        ataacactcgctacttgtgagctttatcactaccaagagtgtgttagaggtaca------    27366
                           ************************************************* *

OK235146-14-4-2021         -acagt------------------------------------------------------    27407
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ON611111-11-5-2022         ------------------------------------------------------------    27450
OP077998-13-7-2022         ------------------------------------------------------------    27440
OP174404-3-7-2022          ------------------------------------------------------------    27267
NC_045512.2-12-2019        -acagtacttttaaaagaaccttgctcttctggaacatacgagggcaattcaccatttca    27533
OV426369-4-12-2021         -acagtacttttaaaagaaccttgctct--------------------------------    27441
OM356217-28-12-2021        cactgacttgctttagcactcaatttgcttttgctt------------------------    27375
ON482431-2-10-2021         -acagtacttttaaaagaaccttgctcttctggctt------------------------    27441
OL835581-22-11-2021        -acagtacttttaaaagaaccttgctcttctgg---------------------------    27398
OK235146-14-4-2021         ---------------------------------------cactcaatttgcttttgcttg    27428
OX031117-16-5-2022         tcctctagctgataacaaatttgcactgacttgctttagcactcaatttgcttttgcttg    27575
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OP174404-3-7-2022          -----------------------t---gacttgctttagcactcaatttgcttttgcttg    27301
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OM356217-28-12-2021        -----------------------------------------------------------g    27376
ON482431-2-10-2021         -----------------------------------------------------------g    27442
OL835581-22-11-2021        ------------------------------------------------------------    27398
OK235146-14-4-2021         tcctgacggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgtt    27488
OX031117-16-5-2022         tcctgacggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgtt    27635
ON611111-11-5-2022         tcctgacggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgtt    27547
OP077998-13-7-2022         tcctgacggcgtaaaacacgtctatcagttacgtgccagatcagtttcacctaaactgtt    27526
OP174404-3-7-2022          tcctgacggcgtaaa---------------------------------------------    27316
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OV426369-4-12-2021         ----------------------------------------tcagcttcacctaaactgtt    27461
OM356217-28-12-2021        tcctgacggcgtaaaacacgtctatcagttacgtgccagatcagcttcacctaaactgtt    27436
ON482431-2-10-2021         tcctgacggcgtaaaacacgtctatcagttacgtgccagatcagcttcacctaaactgtt    27502
OL835581-22-11-2021        ------------------------------------------------aacatacgaggg    27410
OK235146-14-4-2021         catcagacaagaggaagttcaagaactttactctccaatttttcttattgttgcggcaat    27548
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ON611111-11-5-2022         catcagacaagaggaagttcaagaactttactctccaatttttcttattgttgcggcaat    27607
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OV426369-4-12-2021         catcagacaagaggaagttcaagaactttactctccaatttttc---ttgttgcggcaat    27518
OM356217-28-12-2021        catcagacaagaggaagttcaagaactttactctccaatttttcttattgttgcggcaat    27496
ON482431-2-10-2021         catcagacaagaggaagttcaagaactttactctccaatttttcttattgttgcggcaat    27562
OL835581-22-11-2021        catcagacaagaggaagttcaagaactttactctccaatttttcttattgttgcggcaat    27470
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OP077998-13-7-2022         agtgtttataacactttgcttcacactcaaaagaaagacagaatgattgaactttcatta    27646
OP174404-3-7-2022          -------------------------------------acagaatgattgaactttcatta    27373
NC_045512.2-12-2019        agtgtttataacactttgcttcacactcaaaagaaagacagaatgattgaactttcatta    27773
OV426369-4-12-2021         agtgtttataacactttgcttcacactcaaaagaaagatagaatgattgaactttcatta    27578
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ON482431-2-10-2021         agtgtttataacactttgcttcacactcaaaagaaagatagaatgattgaactttcatta    27622
OL835581-22-11-2021        agtgtttataacactttgcttcacactcaaaagaaagatagaatgattgaactttcatta    27530
                                                                * *********************
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Figure 12: *Multi-alignment of selected ORF7a deleted sequences to demonstrate diverse modes of deletions (one, two and three). These sequences were selected BlastN search with full length ORF7a sequence which had three deletions.*

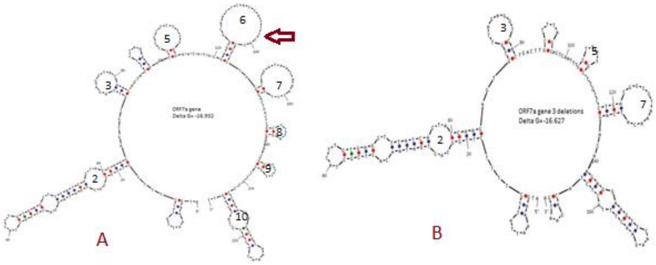
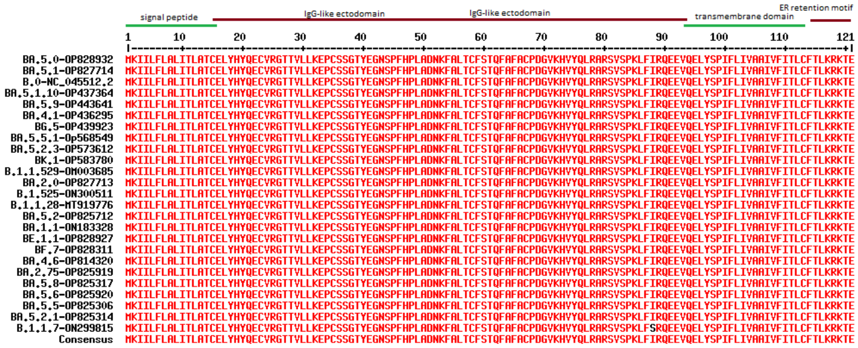


Figure 13: *Demonstration of change in hairpin structure of Corona virus ORF7a gene on three deletions.*



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                     1        10        20        30        40        50        60        70        80        90       100       110      121
                     |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+|
    BA.5.0-OP828932  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.5.1-OP827714  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    B.0-NC_045512.2  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
 BA.5.1.10-OP437364  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.5.9-OP443641  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.4.1-OP436295  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
      BG.5-OP439923  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
  BA.5.5.1-Op568549  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
  BA.5.2.3-OP573612  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
      BK.1-OP583780  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
 B.1.1.529-OM003685  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.2.0-OP827713  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
   B.1.525-ON300511  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
  B.1.1.28-MT919776  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.5.2-OP825712  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.1.1-ON183328  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BE.1.1-OP828927  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
      BF.7-OP828311  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.4.6-OP814320  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
   BA.2.75-OP825919  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.5.8-OP825317  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.5.6-OP825920  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
    BA.5.5-OP825306  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
  BA.5.2.1-OP825314  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
   B.1.1.7-ON299815  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFSRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
          Consensus  MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
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Figure 14: *Multi-alignment of ORF7a protein from diverse COVID-19 variants. We only detected I88S point mutation in ORF7a protein of a B.1.1.7 variant corona virus. But during such data preparation, we detected deletion of ORF7a gene in A.21 variant (ON300492), Q.4 variant (ON299730), B.1.351 variant (ON300512), and B.1.617.2 variant (OM265438) (one sequence each).*

Citation: Chakraborty AK (2022). Dynamics of SARS-Cov-2 ORF7a Gene Deletions and Fate of Downstream ORF7b and ORF8 Genes Expression. SunText Rev Biotechnol 3(1): 142.

The ON940286, ON608606, and ON926042 sequences were omicron BA.2 with spike 24LPP deletion but no 69HV deletion. The OP174404 and OP490708 sequence were omicron BA.4 variant having 141KSF ORF1ab protein deletion (data not shown). The OP791425, OP721043, ON852734, OP753941, OP543868 and OP405203 sequences were likely omicron BA.5 variants. These findings clearly demonstrated the spread of ORF7a deletion mutants into omicron corona virus. Note that all omicron variants had 31ERS N-protein deletion (Figure 10). The accession numbers beginning with OX, OV, OU and OD (UK origin) had no protein expression data. Thus, our protein multi-alignment data was limited. We showed ORF1ab polyprotein deletions that frequently found in different corona virus isolates. The GHVMV, 141KSG and 3675SGF were important deletions and we found ORF7a deletion mutants also had such genetic imprint. Never-the-less, we have no idea regarding the functional consequence in nsp1 transactivator protein for GHVMV and KSF deletion and in nsp6 accessory protein for SGF deletion. Finally, we BlastN searched the full length ORF7a sequence which had three deletions to trap more diverse ORF7a deleted sequences (Figure 11). The accession number OP174404 had first 85nt deletion at 27468, followed by second 45nt deletion at 27608 and a third deletion of 63nt at 27713 position as described before. The ORF7a mutant (accession no. ON482431) had 7nt first deletion at 27468 followed by second 83nt deletion at 27509. Whereas, the ORF7a mutant (accession no. ON611111) had 85nt one deletion at 27468 position and in another mutant (accession no. OP077998) had also one 100nt deletion at the same position. Thus, we showed the dynamics of ORF7a gene deletions with time since 2020 to 2022. It should be noted that few sequences (OV426369, OM356217, and ON482431) were Delta variant with 157FR deletion in the spike whereas one sequence was B.1.1.7 origin with 69HV and 145Y amino acids deletions in the spike. On the other hand, 24LPP deletion only suggested that ON611111 and OX031117 sequences were omicron BA.2 variant which had also 3675SGF ORF1ab deletion. Whereas with 141KSF deletion in nsp1 domain of ORF1ab polyprotein and 24LPP plus 69HV spike deletions suggested BA.4 omicron corona virus (see, accession numbers OP077998 and OP174404). The accession number OK235146 was classified as omicron BA.5 variant with no 141KSF mutation but spike 24LPP plus 69HV spike deletions. But the expression of ORF8 gene was hampered in this virus due to creation of CAA=TAA termination codon and only 26AA length ORF8 might be produced. Anyway, a R52I mutation in the ORF8 gene was also identified in this virus as we had reported earlier (in press). The ORF7a deletion mutants belong to accession numbers OV426369, OM356217, ON482431 and OL835581 were Delta variants with 157FR deletion in the spike but we also found 119DF deletion in the ORF8 gene (data not shown here). Thus, we showed the transmission and propagation of ORF7a deletion mutants across early B.0 and B.1 lineages to B.1.1.7 and B.1.617.2 most deadly variants and then also to mild pathogenic but highly transmissible omicron corona virus variants. We classified omicron variant into sub-variants BA.1, BA.2, BA.4 and BA.5 and found ORF7a deletions in all omicron sub-variants (Figure 12). We showed the hairpin structure of ORF7a gene locus which was changed in three deletion mutants (hairpin loop 6 was abolished) but not in one deletion mutant. We postulated that stiff hairpin loop-2 may have role in driving deletion in the ORF7a gene. We performed a protein multi-alignment of 25 sequences from different corona virus variants and only one I88S point mutation detected. Overall, ORF7a gene deletions found consistently in B.1.1.7 and B.1.617.2 and then B.1.1.529 variants. But point mutation in the ORF7a protein was very limited (Figure 13).

Discussion

The ORF7a deletion B.1 lineage corona viruses were appeared in 2020 and necessary for viral replication and immunity. We showed that the 40-100nt deletions in the ORF7a gene were rampant and transmitted from B.1.1.7 to B.1.617.2 and B.1.1.529 corona viruses. Three deletions in the same gene was limited and we found only two such sequence by searching NCBI database. The ORF7a truncations were defective in suppressing the host immune response, which might explain why these mutations quickly disappeared in the immunocompetent population [22]. The ORF8 is downregulating antigen presentation. Other corona virus accessor protein Nsp6 binds TANK binding kinase 1(TBK1) to supress interferon regulatory factor 3 (IRF3) phosphorylation similar to Nsp13. Such cellular interacting protein binds importin Karyopherin α2 to limit IRF3 nuclear translocation. A Delta variant SARS-CoV-2 originated in Poland with deletion of 872nt ORF7a/b and ORF8 genes (GISAID database accession no. hCoV-19/Poland/PL_ P3686/2021; hCoV-19/Poland/PL_P3688/2021; hCoV19/Poland/PL_P3693/2021). Similar to other RNA viruses, the SARS-CoV-2 genome evolves quite rapidly, indicated by a 1.1 × 103 substitution/site/year mutation rate. Our data clearly presented the propagation of ORF7a deletion mutants in most VOV of corona viruses with or without damaging expression of 43 AA ORF7b protein. The ORF8 protein is also 121 AA and its deregulation is due to creation of termination codon (CAA=TAA; C>T at 27972) with or without S24L mutation but not with L84S mutation (Figure 14). Extensive spike protein mutations occurred in omicron variants and such deletion and point mutation were used to identify different variant type. We clearly demonstrated the ORF7a deletion happened in all corona virus variants including BA.1, BA.2, BA.4 and BA.5 which were spreading now with mild symptoms [23-36].

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Competent Interest

The author declared no conflict of interest. The data was generated by computer analysis of NCBI database.

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