

Table S6. SNPs identified in S-Exon1 viruses at P1', P5', and P10' by deep sequencing.

Passage	Mutation ^a	Codon change	Amino acid change ^b	Location ^c	SNP frequency ^d		
					P1'	P5'	P10'
1', 5', 10'	T 2718 C	<u>G</u> G <u>I</u> → <u>G</u> G <u>C</u>	none	nsp 2	1	0.9969	0.9971
	C 3161 T	<u>G</u> C <u>C</u> → <u>G</u> T <u>C</u>	Ala 148 Val	nsp 3	0.5815	0.1126	0.1174
	A 5723 C	<u>G</u> A <u>G</u> → <u>G</u> C <u>G</u>	Glu 1002 Ala	nsp 3	0.9976	1	0.9949
	C 6536 T	<u>T</u> C <u>A</u> → <u>T</u> T <u>A</u>	Ser 1273 Leu	nsp 3	0.9398	0.9429	0.9383
	A 6700 T	<u>A</u> T <u>G</u> → <u>I</u> T <u>G</u>	Met 1328 Leu	nsp 3	0.9836	0.9713	0.9757
	A 7926 G	<u>G</u> T <u>A</u> → <u>G</u> T <u>G</u>	none	nsp 3	0.998	1	1
	A 12270 T	<u>G</u> T <u>A</u> → <u>G</u> T <u>I</u>	none	nsp 8	0.9838	0.98	0.9779
	A 12374 G	<u>A</u> A <u>C</u> → <u>A</u> G <u>C</u>	Asn 118 Ser	nsp 8	0.9983	1	0.9963
	T 15882 C	<u>I</u> T <u>A</u> → <u>C</u> T <u>A</u>	none	nsp 12	0.9984	0.9974	0.998
	A 15909 G	<u>A</u> T <u>T</u> → <u>G</u> T <u>T</u>	Ile 847 Val	nsp 12	0.9979	0.9971	0.9982
	A 16911 G	<u>A</u> T <u>T</u> → <u>G</u> T <u>T</u>	Ile 249 Val	nsp 13	1	1	0.9972
	A 17222 G	<u>C</u> T <u>A</u> → <u>C</u> T <u>G</u>	none	nsp 13	0.9879	0.979	0.9957
	A 17660 G	<u>G</u> A <u>A</u> → <u>G</u> A <u>G</u>	none	nsp 13	0.9956	1	0.9933
	A 18042 T	<u>A</u> C <u>A</u> → <u>I</u> C <u>A</u>	Thr 25 Ser	nsp 14	0.989	0.989	0.9817
	T 20382 C	<u>T</u> A <u>C</u> → <u>C</u> A <u>C</u>	Tyr 278 His	nsp 15	0.1951	0.9428	0.9897
	T 22697 C	<u>A</u> C <u>I</u> → <u>A</u> C <u>C</u>	none	Spike	0.9948	0.9965	0.997
	C 23412 T	<u>C</u> A <u>T</u> → <u>T</u> A <u>T</u>	His 641 Tyr	Spike	0.1412	0.969	0.9865
	T 25281 C	<u>A</u> T <u>G</u> → <u>A</u> C <u>G</u>	Met 5 Thr	ORF 3a	0.9953	1	1
	C 26679 T	<u>T</u> A <u>C</u> → <u>T</u> A <u>I</u>	none	M	0.973	0.974	0.9876
	A 26896 G	<u>A</u> T <u>C</u> → <u>G</u> T <u>C</u>	Ile 167 Val	M	0.9974	0.9957	0.976
T 27194 C	<u>I</u> T <u>T</u> → <u>C</u> T <u>T</u>	Phe 41 Leu	ORF 6	1	0.9982	1	
T 27476 C	<u>G</u> C <u>I</u> → <u>G</u> C <u>C</u>	none	ORF 7a	0.9957	0.9914	0.9924	
A 29109 T	<u>A</u> C <u>A</u> → <u>A</u> C <u>T</u>	none	N	0.9919	0.9955	0.9705	
1', 5'	T 19283 C	<u>A</u> A <u>T</u> → <u>A</u> A <u>C</u>	none	nsp 14	0.2378	0.0727	
1', 10'	C 2569 T	<u>C</u> C <u>C</u> → <u>T</u> C <u>C</u>	Pro 589 Ser	nsp 2	0.12		0.0958
5', 10'	G 1813 A	<u>G</u> T <u>A</u> → <u>A</u> T <u>A</u>	Val 337 Ile	nsp 2		0.2362	0.8339
	A 11790 G	<u>G</u> T <u>A</u> → <u>G</u> T <u>G</u>	none	nsp 7		0.1788	0.9016
	A 17472 T	<u>A</u> T <u>G</u> → <u>I</u> T <u>G</u>	Met 436 Leu	nsp 13		0.0741	0.8947
	A 21191 G	<u>T</u> C <u>A</u> → <u>T</u> C <u>G</u>	none	nsp 16		0.1603	0.9291
	A 23604 G	<u>A</u> C <u>T</u> → <u>G</u> C <u>T</u>	Thr 705 Ala	Spike		0.2272	0.9069
	T 26226 A	<u>C</u> I <u>T</u> → <u>C</u> A <u>T</u>	Leu 37 His	E		0.276	0.9492
	G 26781 A	<u>G</u> T <u>G</u> → <u>G</u> T <u>A</u>	none	M		0.1622	0.9375
	T 28572 C	<u>A</u> A <u>T</u> → <u>A</u> A <u>C</u>	none	N		0.0977	0.7783
1'	C 288 T	<u>G</u> T <u>C</u> → <u>G</u> T <u>I</u>	none	nsp 1	0.1036		
	T 6360 C	<u>C</u> C <u>T</u> → <u>C</u> C <u>C</u>	none	nsp 3	0.5773		
	T 6744 C	<u>A</u> C <u>I</u> → <u>A</u> C <u>C</u>	none	nsp 3	0.1338		
	T 10266 C	<u>T</u> C <u>I</u> → <u>T</u> C <u>C</u>	none	nsp 5	0.1309		
	T 14319 C	<u>I</u> T <u>T</u> → <u>C</u> T <u>T</u>	Phe 317 Leu	nsp 12	0.2129		
	T 14767 C	<u>A</u> T <u>C</u> → <u>A</u> C <u>C</u>	Ile 466 Thr	nsp 12	0.0762		
	T 16712 A	<u>T</u> T <u>T</u> → <u>T</u> T <u>A</u>	Phe 182 Leu	nsp 13	0.0671		
	T 17397 C	<u>I</u> T <u>G</u> → <u>C</u> T <u>G</u>	none	nsp 13	0.09		
	T 20861 C	<u>A</u> C <u>I</u> → <u>A</u> C <u>C</u>	none	nsp 16	0.1169		
	C 21588 T	<u>C</u> A <u>T</u> → <u>I</u> A <u>T</u>	His 33 Tyr	Spike	0.0737		
	T 24703 A	<u>T</u> I <u>C</u> → <u>T</u> A <u>C</u>	Phe 1071 Tyr	Spike	0.0864		
	T 25729 C	<u>T</u> A <u>I</u> → <u>T</u> A <u>C</u>	none	ORF 3a	0.2634		
			<u>A</u> T <u>G</u> → <u>A</u> C <u>G</u>	Met 14 Thr	ORF 3b		
	G 25895 A	<u>G</u> T <u>T</u> → <u>A</u> T <u>T</u>	Val 210 Ile	ORF 3a	0.0975		
		<u>A</u> A <u>G</u> → <u>A</u> A <u>A</u>	none	ORF 3b			
T 26802 C	<u>A</u> G <u>T</u> → <u>A</u> G <u>C</u>	none	M	0.1366			
T 27837 C	<u>G</u> T <u>A</u> → <u>G</u> C <u>A</u>	Val 20 Ala	ORF 8a	0.6885			
5'	T 11124 C	<u>T</u> C <u>I</u> → <u>T</u> C <u>C</u>	none	nsp 6		0.1765	
	G 13847 T	<u>A</u> A <u>G</u> → <u>A</u> A <u>I</u>	Lys 159 Asn	nsp 12		0.0775	
	T 26354 C	n.a.	n.a.	IGR		0.0685	
10'	T 1383 C	<u>T</u> G <u>T</u> → <u>T</u> G <u>C</u>	none	nsp 2			0.1061
	C 2626 T	<u>C</u> T <u>C</u> → <u>I</u> T <u>C</u>	Leu 608 Phe	nsp 2			0.112
	T 2949 C	<u>G</u> G <u>I</u> → <u>G</u> G <u>C</u>	none	nsp 3			0.6963
	T 3386 C	<u>A</u> T <u>A</u> → <u>A</u> C <u>A</u>	Ile 223 Thr	nsp 3			0.1189
	T 4608 A	<u>C</u> C <u>T</u> → <u>C</u> C <u>A</u>	none	nsp 3			0.1072
	T 4657 C	<u>T</u> A <u>C</u> → <u>C</u> A <u>C</u>	Tyr 647 His	nsp 3			0.0706
	G 5445 A	<u>G</u> T <u>G</u> → <u>G</u> T <u>A</u>	none	nsp 3			0.0908
	T 12895 C	<u>I</u> T <u>A</u> → <u>C</u> T <u>A</u>	none	nsp 9			0.0552
	A 12928 G	<u>A</u> G <u>T</u> → <u>G</u> G <u>T</u>	Ser 105 Gly	nsp 9			0.6579

A 18940 G	<u>GAT</u> → <u>GGT</u>	Asp 324 Gly	nsp 14	0.7004
T 21092 A	<u>GCT</u> → <u>GCA</u>	none	nsp 16	0.1457
A 22816 C	<u>TAT</u> → <u>TCT</u>	Tyr 442 Ser	Spike RBM	0.0502
T 23656 C	<u>ATG</u> → <u>ACG</u>	Met 722 Thr	Spike	0.3364
G 24091 A	<u>GGA</u> → <u>GAA</u>	Gly 867 Glu	Spike	0.0856
A 26312 G	<u>AAC</u> → <u>GAC</u>	Asn 66 Asp	E	0.2145
T 27144 C	<u>ATT</u> → <u>ACT</u>	Ile 24 Thr	ORF 6	0.0847
C 28566 T	<u>ACC</u> → <u>ACT</u>	none	N	0.0935

^a **Red**, mutations in all 3 passages; **purple**, shared in P1' and P5' but not P10'; **orange**, shared in P1' and P10' but not P5'; **green**, shared in P5' and P10' but not P1'; **blue**, mutations unique to a single passage.

^b Amino acid positions within nsps refer to location within the respective mature nsp. 'None' indicates synonymous changes.

^c IGR, intergenic region; RBM, receptor binding motif.

^d Proportion of reads containing each SNP at the passage indicated. SNP frequencies of ≥ 0.05 are shown.