Supplementary Materials

Generation of SARS-CoV-2 escape mutations by monoclonal antibody therapy

Manon Ragonnet-Cronin[1,2*#], Rungtiwa Nutalai[3*], Jiandong Huo[4*#], Aiste Dijokaite-Guraliuc[3*], Raksha Das[3], Aekkachai Tuekprakhon[3], Piyada Supasa[3], Chang Liu[3,5], Muneeswaran Selvaraj[3], Natalie Groves[1], Hassan Hartman[1], Nicholas Ellaby[1], J. Mark Sutton[1], Mohammad W. Bahar[4], Daming Zhou[4,5], Elizabeth Fry[4], Jingshan Ren[4], Colin Brown[1], Paul Klenerman[6,7,8,9], Susanna J. Dunachie[6,7,9], Juthathip Mongkolsapaya[3,10],Susan Hopkins[1], Meera Chand[1], David I. Stuart[4#^], Gavin R. Screaton[3#*] and Sakib Rokadiya[1#*]

1. Genomics Public Health Analysis, UK Health Security Agency

2. Centre for Global Infectious Disease Analysis, Imperial College London

3. Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, UK

4. Division of Structural Biology, Nuffield Department of Medicine, University of Oxford, The Wellcome Centre for Human Genetics, Oxford, UK

5. Chinese Academy of Medical Science (CAMS) Oxford Institute (COI), University of Oxford, Oxford, UK

6. Nuffield Department of Medicine, University of Oxford, Oxford, UK

7. Oxford University Hospitals NHS Foundation Trust, Oxford, UK

8. Translational Gastroenterology Unit, University of Oxford, UK

9. NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK

10. Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, Department of Medicine, University of Oxford, Oxford, UK

Figure S1. P-values for differences in spike (S) amino acid frequencies between pre- and post-treatment sequences. The indicated cut off dates, following the mAb treatment were used for the acquisition of the post treatment sample. p values were calculated using a one-sided Fisher's test. No adjustments were made for multiple comparisons. All exact p values are provided in the source data for this figure.

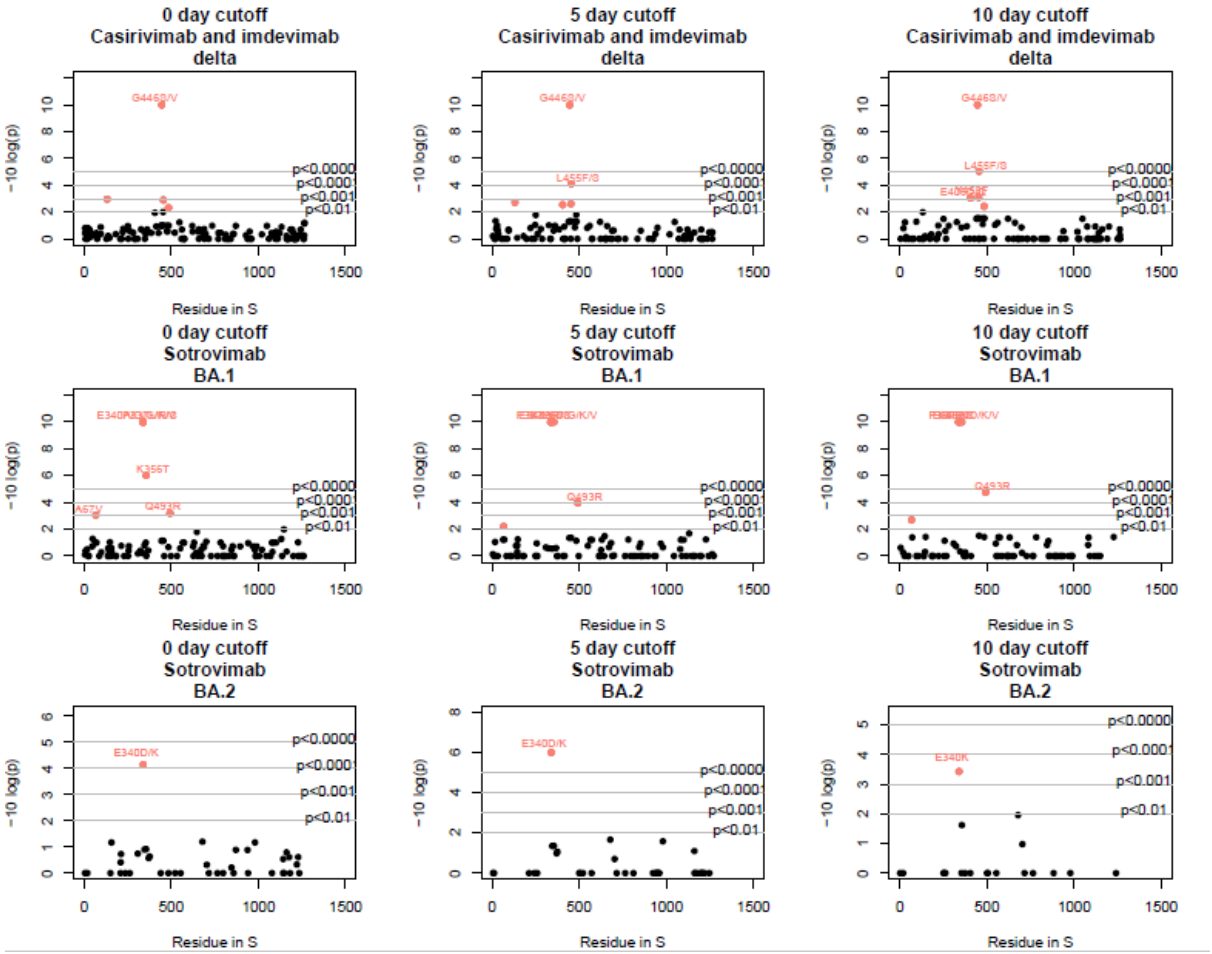

Figure S2. Surface plasmon resonance (SPR) analysis of interaction between Delta and BA.1 RBD mutants and therapeutic mAbs. (A-D; F-G; I, L, N, P) Sensorgrams showing the binding of wild-type Delta RBD and Delta RBD mutants to casirivimab/imdevimab, with affinity and kinetic parameters shown. (E, J, K, M, O) 1:1 binding equilibrium analysis of binding of Delta RBD mutants to casirivimab/imdevimab, with affinity values shown. (H) Binding of Delta RBD+G446V to imdevimab is severely reduced compared to that of wild-type Delta RBD, so that the binding could not be

accurately determined, as shown by a single-injection of 1 µM RBD over sample flow cells containing imdevimab. Related to **Figure S3** and **Table 3**.

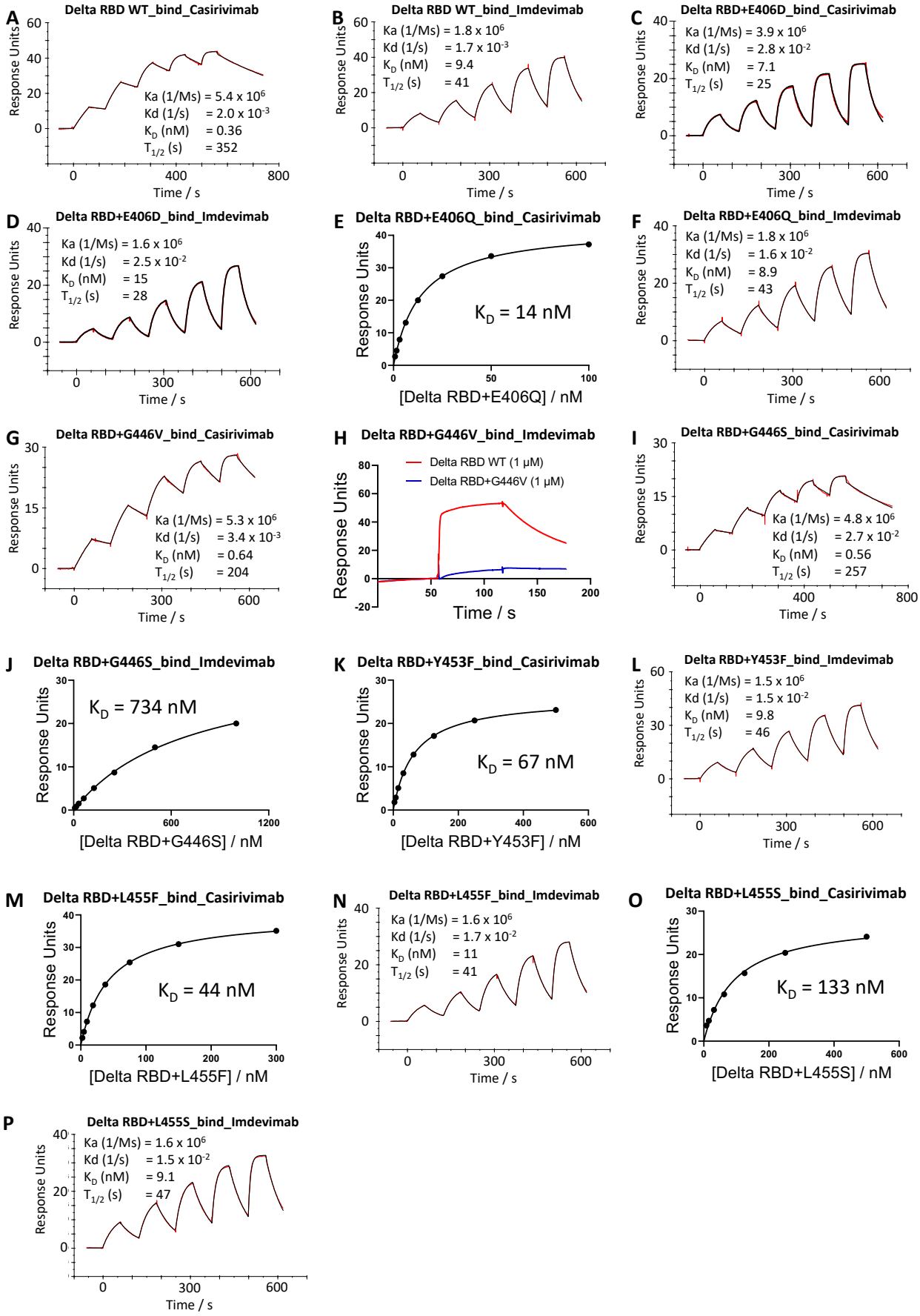

Figure S3. Surface plasmon resonance (SPR) analysis of interaction between Delta and BA.1 RBD mutants and therapeutic mAbs. (A, C) 1:1 binding equilibrium analysis of binding of Delta RBD mutants to casirivimab, with affinity values shown. (B, D) Binding of Delta RBD+G446V+Y453F and Delta RBD+G446V+L455F to imdevimab is severely reduced compared to that of wild-type Delta RBD, so that the binding could not be accurately determined, as shown by a single-injection of 1 µM RBD over sample flow cells containing imdevimab. (E) Sensorgram showing the binding of wild-type BA.1 RBD to sotrovimab, with affinity and kinetic parameters shown (published in Dejnirattisai et al., 2022). (F-K) 1:1 binding equilibrium analysis of binding of BA.1 RBD mutants to sotrovimab, with affinity values shown. Related to **Figure S2** and **Table 3**.

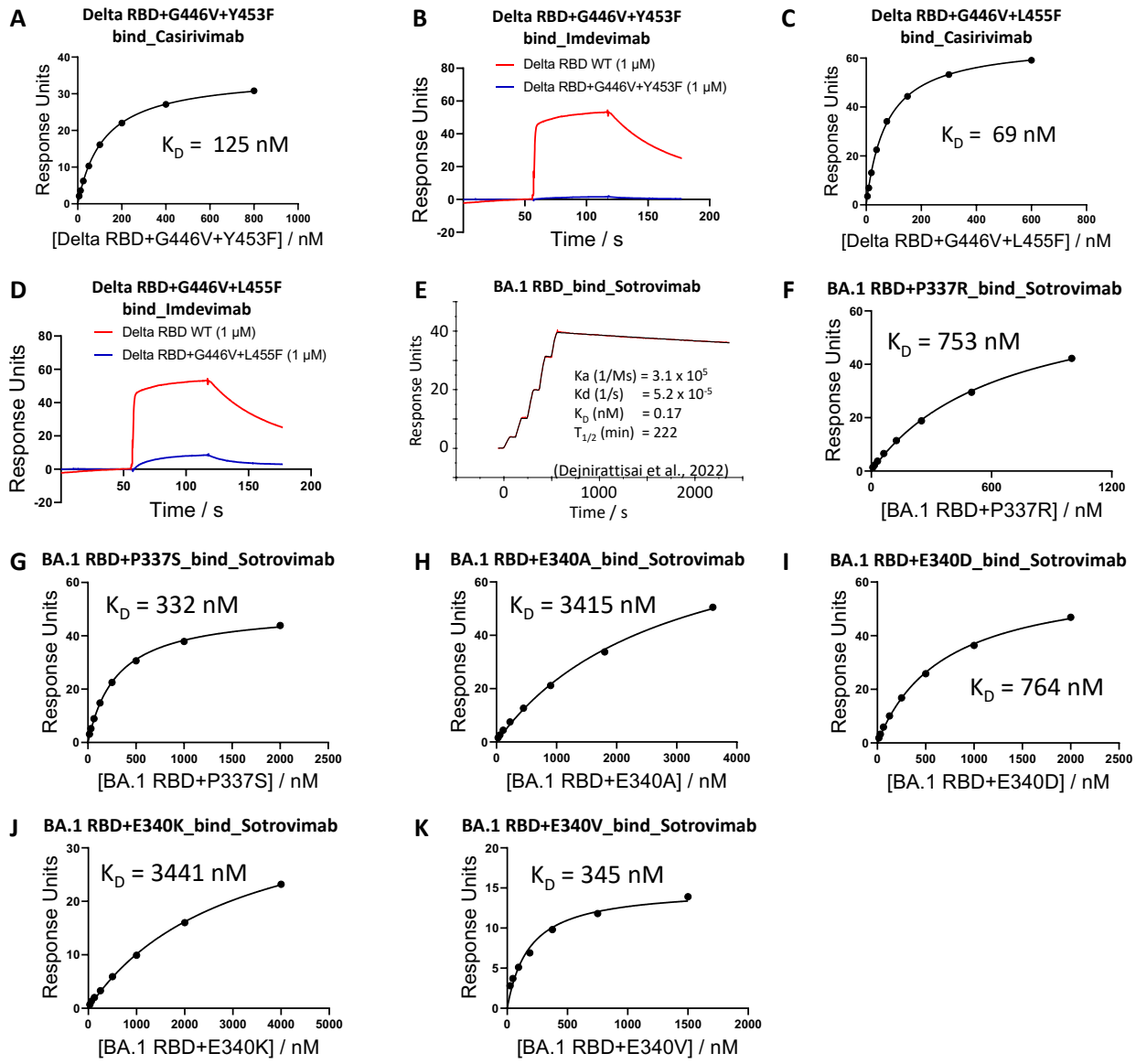

Table S1. Dataset sizes (number of sequences). Note that some patients received multiple courses of treatment and thus may be counted more than once in the table.

Treatment	Variant	Number of sequences (pre-treatment)	Number of sequences (≥ 1 day post-treatment)	Number of sequences (≥ 5 day post-treatment)	Number of sequences (≥ 10 day post-treatment)
Casirivimab and imdevimab	BA.1	181	116	94	80
Casirivimab and imdevimab	BA.2	0	14	14	14
Casirivimab and imdevimab	delta	2173	298	138	82
Molnupiravir	BA.1	1642	181	109	70
Molnupiravir	BA.2	285	17	11	8
Molnupiravir	delta	29	8	7	3
Paxlovid	BA.1	329	19	8	4
Paxlovid	BA.2	698	41	15	10
Paxlovid	delta	0	2	2	2
Remdesivir	BA.1	1268	529	371	307
Remdesivir	BA.2	249	106	83	75
Remdesivir	delta	4779	955	402	224
Sotrovimab	BA.1	3959	531	318	211
Sotrovimab	BA.2	1656	130	49	25
Sotrovimab	delta	36	8	6	3

Table S2. Treatment/gene/variant combinations tested

Treatment	Gene	Variants
Sotrovimab	*spike*	Delta, BA.1, BA.2
Casirivimab and imdevimab	*spike*	Delta, BA.1, BA.2
Remdesivir	*nsp7, nsp8, nsp9, nsp10, nsp12*	Delta, BA.1, BA.2
Molnupiravir	*nsp7, nsp8, nsp9, nsp10, nsp12*	Delta, BA.1, BA.2
Paxlovid	*nsp5*	Delta, BA.1, BA.2

Table S3. Template, primers and expression vectors used for cloning of each RBD.

RBD construct	Template (pseudovirus plasmid)	PCR primer sequence (5'to 3')	vector
Delta+E406D	Delta+E406D	F: CAGCTCCTGGGCAACGTGCT R: CGTAAAAGGAGCAACATAG	pNEO
Delta+E406Q	Delta+E406Q		
Delta+G446S	Delta+G446S		
Delta+G446V	Delta+G446V		
Delta+Y453F	Delta+Y453F		
Delta+L455F	Delta+L455F		
Delta+L455S	Delta+L455S		
Delta+G446V+Y453F	Delta+G446V+Y453F		
BA.1+P337R	BA.1+P337R		
BA.1+P337S	BA.1+P337S		
BA.1+E340A	BA.1+E340A		
BA.1+E340D	BA.1+E340D		
BA.1+E340K	BA.1+E340K		
BA.1+E340V	BA.1+E340V		
BA.1+K356T	BA.1+K356T		
BA.1+R493Q	BA.1+R493Q		

Table S4. Primers for pseudoviruses

Primer	Sequence (5'to 3')
Delta variants	
Delta+E406D_F	GACAGCTTCGTGATCAGAGGCGACGACGTGAGACAGATCGCGCCAGGG
Delta+E406D_R	CCCTGGCGCGATCTGTCTCACGTCGTCGCCTCTGATCACGAAGCTGTC
Delta+E406Q_F	GACAGCTTCGTGATCAGAGGCGACCAAGTGAGACAGATCGCGCCAGGG
Delta+E406Q_R	CCCTGGCGCGATCTGTCTCACTTGGTCGCCTCTGATCACGAAGCTGTC
Delta+G446S_F	GAACTCTAACAATCTAGATTCGAAAGTTAGCGGCAATTACAATTACCTGTAC
Delta+G446S_R	GTACAGGTAATTGTAATTGCCGCTAACTTTCGAATCTAGATTGTTAGAGTTC
Delta+G446V_F	TAACAATCTAGATTCGAAAGTTGTAGGCAATTACAATTACCTGTAC
Delta+G446V_R	GTACAGGTAATTGTAATTGCCTACAACTTTCGAATCTAGATTGTTA
Delta+Y453F_F	GGCAATTACAATTACCGGTTCAGACTGTTCAGAAAGAGC
Delta+Y453F_R	GCTCTTTCTGAACAGTCTGAACCGGTAATTGTAATTGCC
Delta+L455F_F	GGCAATTACAATTACCGGTACAGATTCTTCAGAAAGAGCAATCTGAAGCC
Delta+L455F_R	GGCTTCAGATTGCTCTTTCTGAAGAATCTGTACCGGTAATTGTAATTGCC
Delta+L455S_F	GGCAATTACAATTACCGGTACAGAAGCTTCAGAAAGAGCAATCTGAAGCC
Delta+L455S_R	GGCTTCAGATTGCTCTTTCTGAAGCTTCTGTACCGGTAATTGTAATTGCC
Delta+G446V+Y453F_F	GTAGGCAATTACAATTACCGGTTCAGACTGTTCAGAAAGAGC
Delta+G446V+Y453F_R	GAACCGGTAATTGTAATTGCCTACAACTTTCGAATCTAGATTG
BA.1 variants	
BA.1+P337R_F	ATCACCAATCTGTGCCGTTTCGACGAGGTGTTC
BA.1+P337R_R	CCTCGTCGAAACGGCACAGATTGGTGATATTAG
BA.1+P337S_F	TCACCAATCTGTGCAGTTTCGACGAGGTGTTCAATG
BA.1+P337S_R	CACCTCGTCGAAACTGCACAGATTGGTGATATTAG
BA.1+E340A_F	CTGTGCCCTTTCGACGCGGTGTTCAATGCCAC
BA.1+E340A_R	GTGGCATTGAACACCGCGTCGAAAGGGCACAG
BA.1+E340D_F	GTGCCCTTTCGACGATGTGTTCAATGCCACC
BA.1+E340D_R	GGTGGCATTGAACACATCGTCGAAAGGGCAC
BA.1+E340K_F	CAATCTGTGCCCTTTCGACAAGGTGTTCAATGCCAC
BA.1+E340K_R	GTGGCATTGAACACCTTGTCGAAAGGGCACAGATTG
BA.1+E340V_F	GCGTAGCTGAAACCGGCACCAATCTGTGCCCTTTCGACGTGGTGTTCAATGCCACCAG
BA.1+E340V_R	CTGGTGGCATTGAACACCACGTCGAAAGGGCACAGATTGGTGCCGGTTTCAGCTACGC
BA.1+K356T_F	GCCAGCGTGTACGCATGGAACCGCACCCGGATAAGCAATTGCGTGGCC
BA.1+K356T_R	GGCCACGCAATTGCTTATCCGGGTGCGGTTCCATGCGTACACGCTGGC
BA.1+R493Q_F	GGCTTCAATTGCTACTTCCCTCTGCAGAGCTACTCGTTCAGACCTACC
BA.1+R493Q_R	GGTAGGTCTGAACGAGTAGCTCTGCAGAGGGAAGTAGCAATTGAAGCC
pcDNA3.1 vector	
pcDNA3.1_BamHI_F	GGATCCATGTTCCTGCTGACCACCAAGAG
pcDNA3.1_Tag_S_EcoRI_R	GAATTCTCACTTCTCGAACTGAGGGTGGC
pcDNA3.1_Tag_S_EcoRI_F	GCCACCCTCAGTTCGAGAAGTGAGAATTC
pcDNA3.1_BamHI_R	CTCTTGGTGGTCAGCAGGAACATGGATCC