Mutation panel of SARS-CoV-2 Alpha, Gamma and Eta variants in wastewaters in Italy detected by nanopore technology long-read amplicon sequencing La Rosa G., Brandtner D., Mancini P., Veneri C., Bonanno Ferraro G., Bonadonna L., Lucentini L., Suffredini E.
Water

SUPPLEMENTARY MATERIAL

Table S1. Results of real-time RT-qPCR detection of SARS-CoV-2 in the tested samples

Sample ID	Recovery (%)	g.c. / μL of RNA	g.c. / L of sewage	PCR ID 980
4158	1.71%	6.09	1.52×10^4	+
4159	1.28%	6.04	1.51×10^4	+
4160	5.31%	2.40	6.00×10^3	-
4161	2.52%	not detected	-	-
4162	1.61%	5.09	1.27×10^4	+
4163	2.11%	36.26	9.06×10^4	+
4170	2.12%	3.50	8.75×10^3	-
4171	2.35%	2.11	5.27×10^3	-
4172	3.06%	19.39	4.85×10^4	+
4173	1.91%	27.77	6.94×10^4	+
4174	6.16%	1.07	2.68×10^3	-
4175	4.52%	0.78	1.94×10^3	-

Table S2. Number of raw and final reads selected after filtering steps

Sample ID	Raw Data	dual-barcode filtering	HiFi (q 8. trim end 20) + 1400-1700 nt	Aligned to SARS-CoV-2 reference sequence NC_045512
BC01	1367887	370776	312766	312697
BC02	1925893	668332	328851	328701
BC03	764517	188387	331399	330950

Table S3. Concatenated mutations queried using the BAMQL tool v1.6 for the detection of each VOC/VOI

Variant	Mutations (aa substitutions or deletions)	Queries
Alpha	69-70del	21764.A and 21771.T and exclude(21765.T. 21769.T)
	144del	21990.T and 21994.T and exclude(21992.T)
	N501Y	23063.T
	A570D	23271.A
Beta	D80A	21801.C
	D215G	22206.G
	K417N	22813.T
	E484K	23012.A
	N501Y	23063.T
Gamma	D138Y	21794.T
	R190S	22132.T
	K417T	22812.C
	E484K	23012.A
	N501Y	23063.T
Delta	156-157-158del	22028.G and 22035.G and exclude(22029.A. 22034.A)
	L452R	22917.G
	T478K	22995.A
Eta	A67V	21762.T
	69-70del	21764.A and 21771.T and exclude(21765.T. 21769.T)
	144del	21990.T and 21994.T and exclude(21992.T)
	E484K	23012.A

Numbers represent nucleotide positions referred to SARS-CoV-2 reference genome NC_045512. Reads were queried for concatenated mutations by: a) selecting defined nucleotide mutations; b) for deletions, by searching for the absence of a wild type nucleotide within the deleted region, in combination with the presence of the wild type nucleotide upstream (5') and downstream (3') of the deletion. This latter search criterion was included to minimize selection of random deletions generated by nanopore sequencing.

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Figure S1. AlleleClusterSummary for pool BC01 (Clustering unsupervised results)

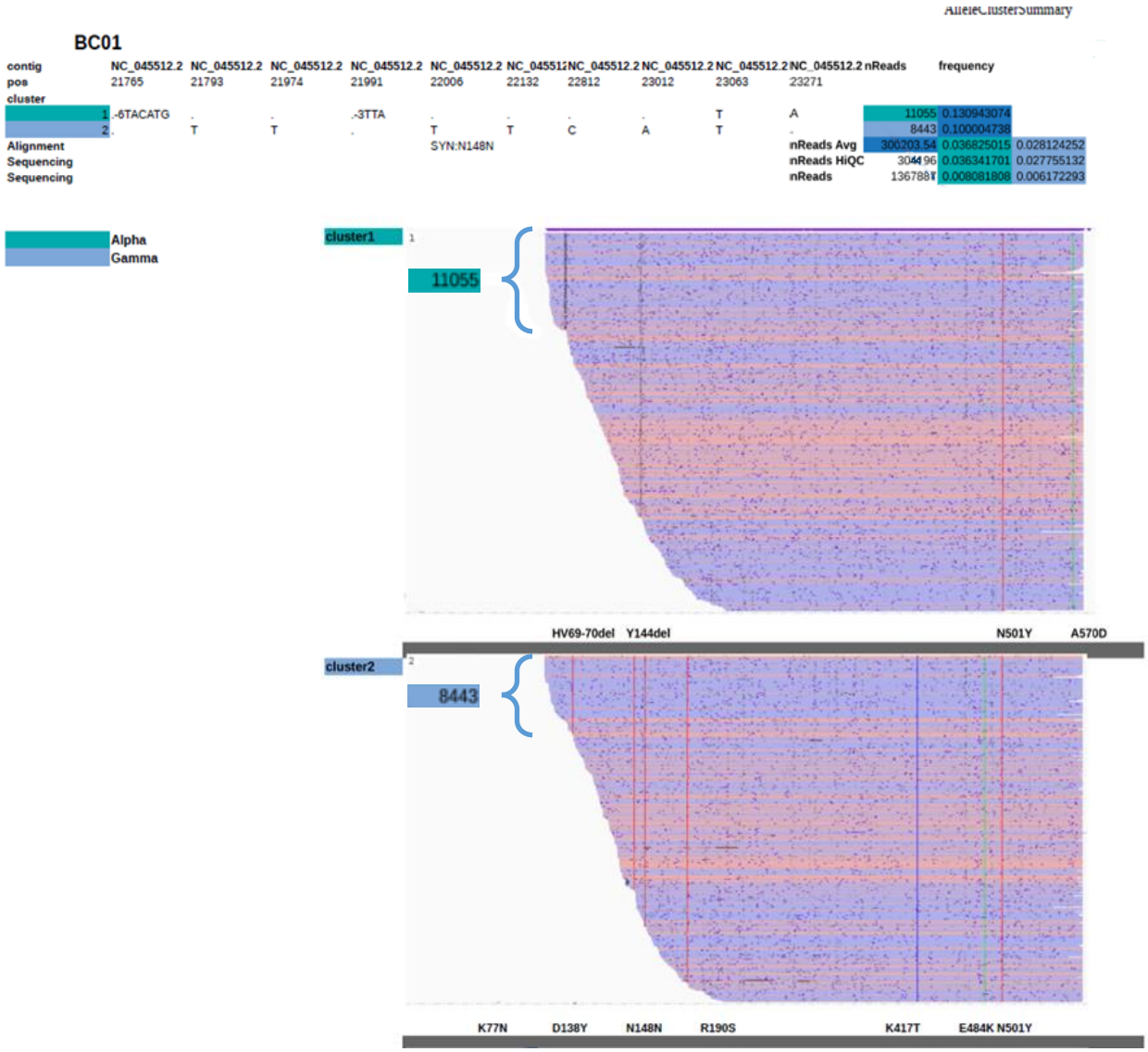


Figure S2. Mutations detected in the alpha cluster

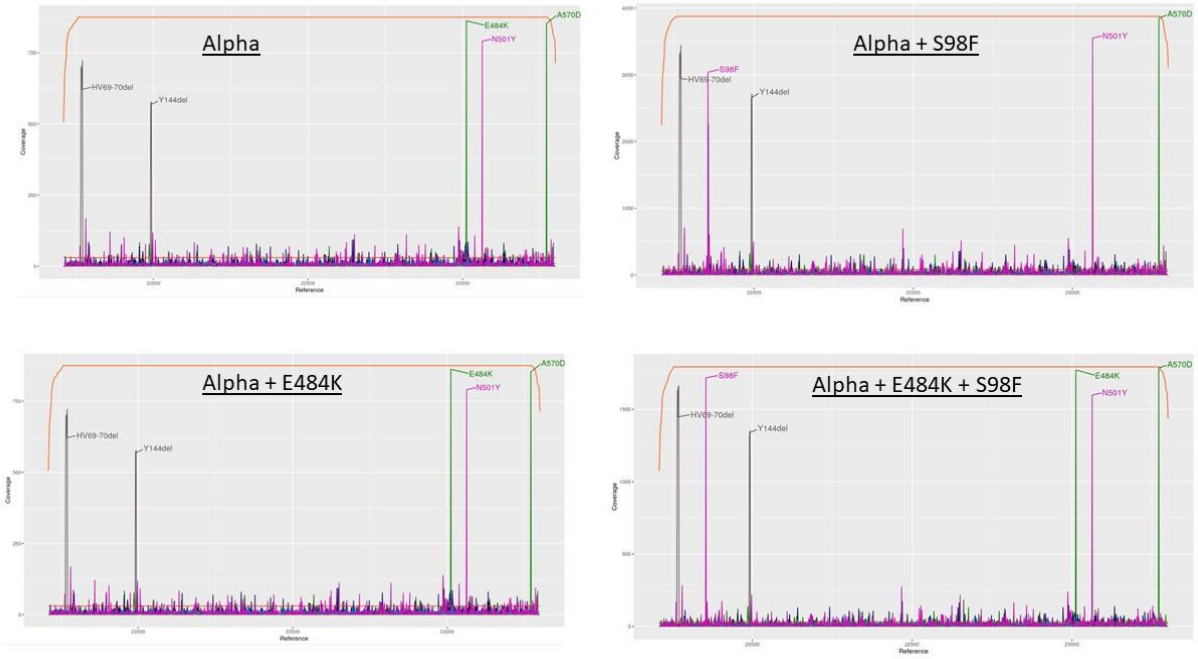
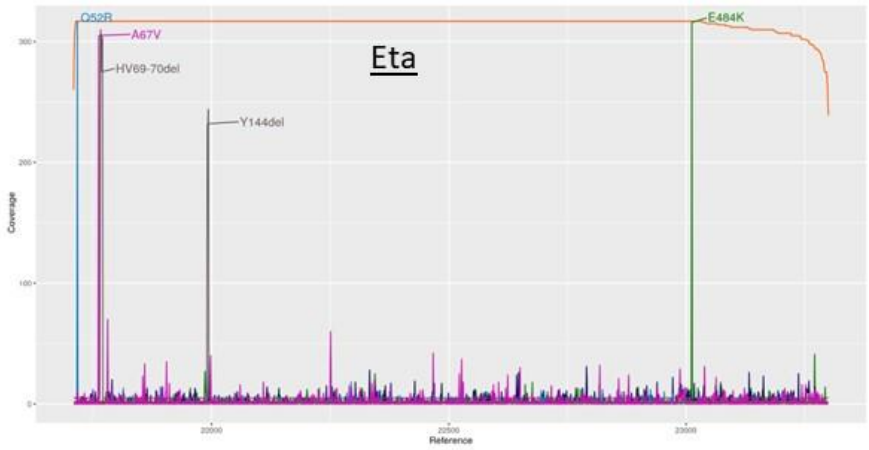


Figure S3. Mutations detected in the eta cluster



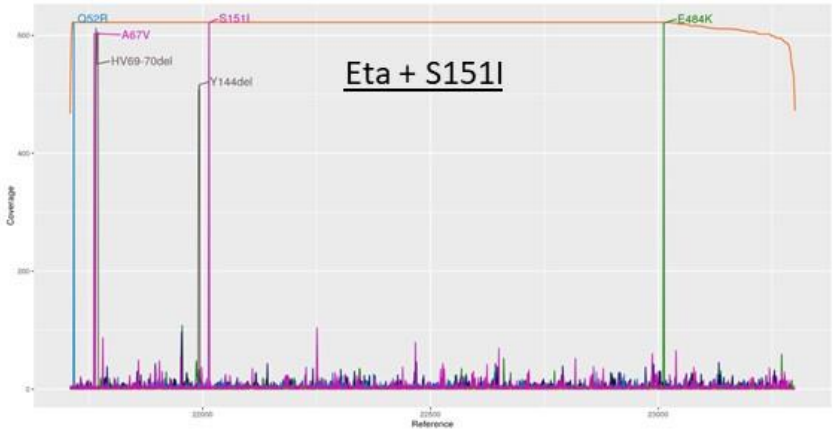


Figure S4. Venn diagrams for Alpha and Eta reads in pool BC02

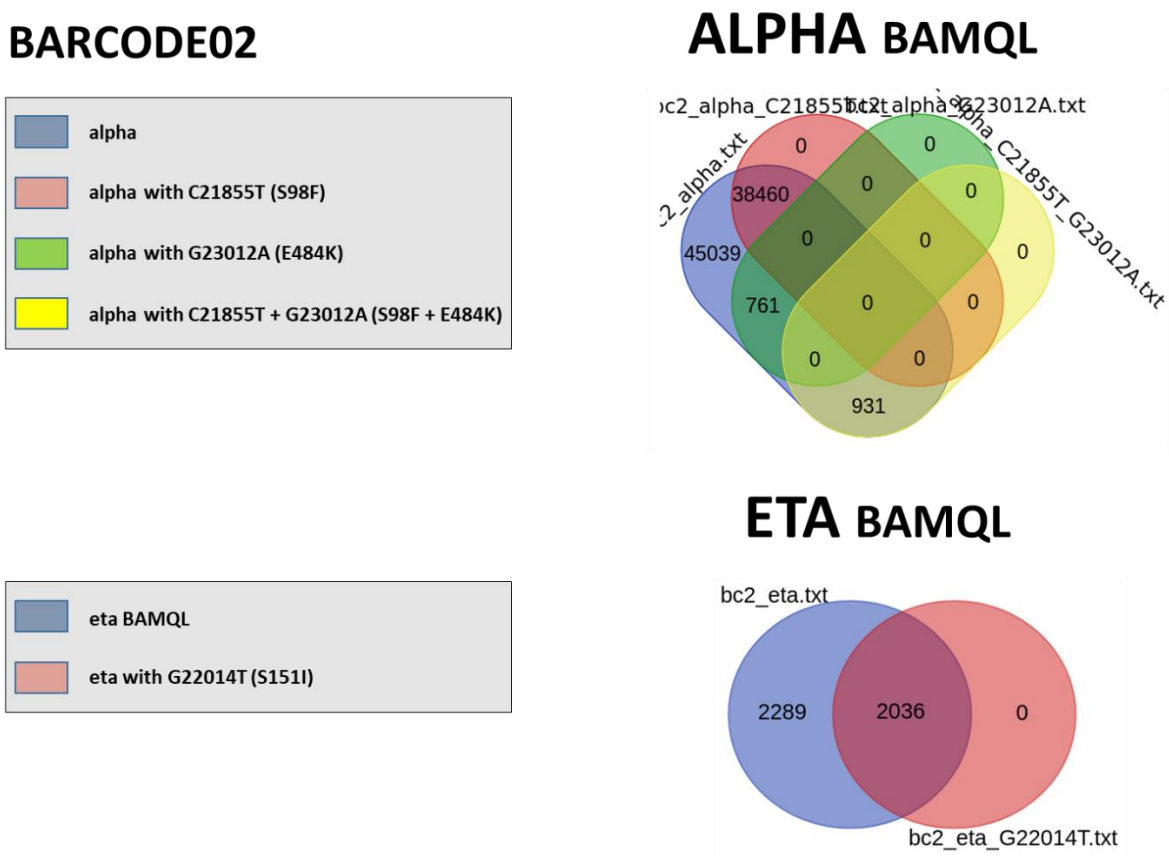
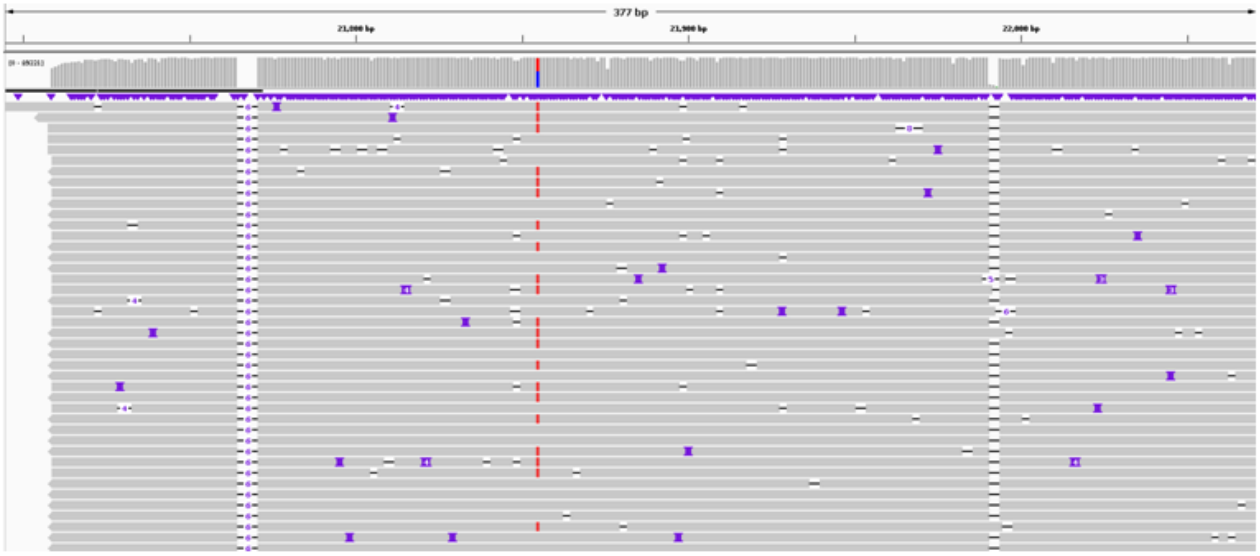


Figure S5. Distribution of small deletions in assigned reads (A) and in unassigned reads (B): small deletions are significantly more frequent in unassigned reads

(Snapshots from IGV alignment viewer)

A



B

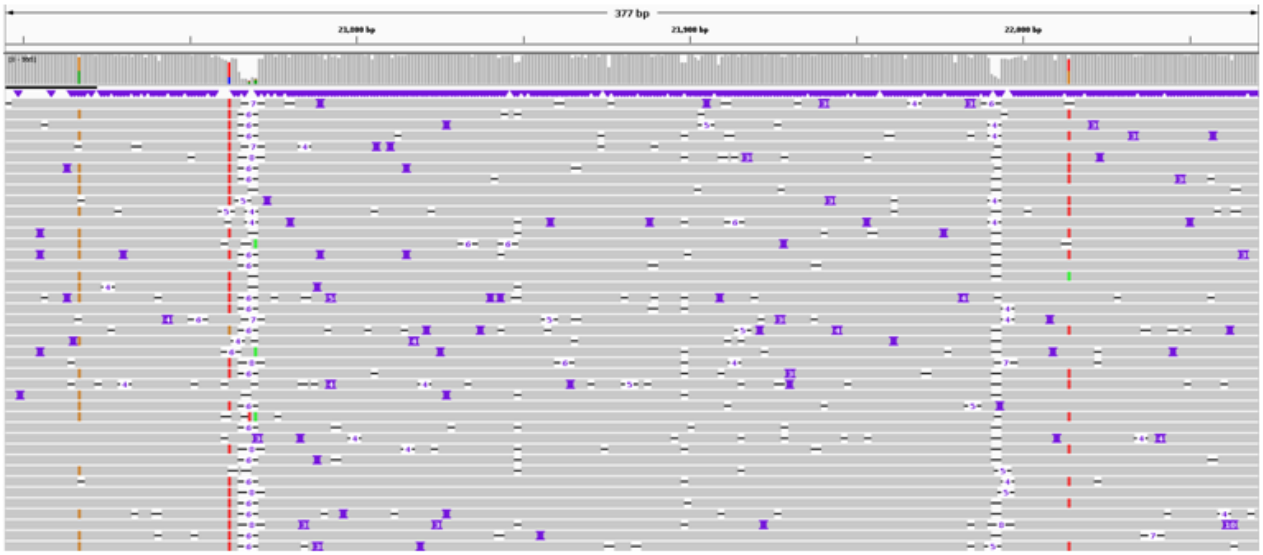


Figure S6. Insertion/Deletion analysis in assigned reads (A) and unassigned reads (B): in unassigned reads Ins/Del ratio is exceedingly low in the range of 10 bases indicating an abundance of small deletions in comparison to assigned reads