Evidence for SARS-CoV-2 Delta and Omicron co-infections and recombination

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Abstract

Between November 2021 and February 2022, SARS-CoV-2 Delta and Omicron variants co-circulated in the United States, allowing for co-infections and possible recombination events. We sequenced 29,719 positive samples during this period and analyzed the presence and fraction of reads supporting mutations specific to either the Delta or Omicron variant. Our sequencing protocol uses hybridization capture and is thus less subject to artifacts observed in amplicon-based approaches that may lead to spurious signals for recombinants. We identified 20 co-infections, one of which displayed evidence of a low recombinant viral population. We also identified two independent cases of infection by a Delta-Omicron recombinant virus, where 100% of the viral RNA came from one clonal recombinant. In both cases, the 5´-end of the viral genome was from the Delta genome, and the 3´-end from Omicron, though the breakpoints were different. Delta-Omicron recombinant viruses were rare, and there is currently no evidence that the two Delta-Omicron recombinant viruses identified are more transmissible between hosts compared to the circulating Omicron lineages.

Introduction

Recombination is one way a virus can evolve to acquire a new combination of mutations. Recombinations have played an important role in the evolution of the RNA viruses HIV-1 and SARS-CoV-2 (Fischer et al., 2021). In humans, the analysis of the first 87,695 SARS-CoV-2 genomes shared on GISAID in 2021 identified 225 sequences of likely recombinant origins (Varabyou et al., 2021). However, tracking recombinations for SARS-CoV-2 remains challenging because of the relatively low diversity of the genomes compared to the large number of genomes sequenced and shared publicly. Moreover, some sequences may look like recombinations, but in reality they are more likely to be due to contamination, technical artifacts, or naturally occurring mutations shared by multiple variants.

In early November 2021, the Omicron variant was first detected by scientists in Botswana and South Africa and rapidly spread across the globe (Viana et al., 2022). As of March 2022, the Omicron variant includes the three Pango lineages BA.1 (Nextstrain clade 21K), BA.2 (Nextstrain clade 21L) and BA.3, and is characterized by a large number of mutations in the spike protein (Viana et al., 2022). In the United States, the first Omicron case was reported on December 1 (by a team at University of California San Francisco, reported by press release) based on the sequencing of a sample collected a few days earlier. At that time, the Delta variant was the dominant variant in the United States (Bolze et al., 2021; Earnest et al., 2021), which led to a period where the Delta and Omicron variants were co-circulating. We hypothesized that a period of co-circulation of two distinct variants would lead to some cases of co-infections, and later result in the emergence of a new SARS-CoV-2 variant resulting from the recombination of a Delta variant and an Omicron variant. This, in turn, would result in a new combination of mutations with unknown properties.

The aims of this study were (i) to look for cases co-infected with Delta and Omicron variants, and (ii) to identify cases infected by a Delta and Omicron recombinant in the United States.

Results

Co-circulation of Delta and Omicron variants in the United States

We sequenced and assigned a lineage to 29,719 samples positive for SARS-CoV-2 collected by the Helix laboratory across the United States (**Table S1**) between 22 November 2021 and 13 February 2022 for genomic surveillance purposes. These samples came from anterior nasal swabs of different individuals, with one viral sequence assay performed per person infected (similar to a cross-sectional analysis). The large majority of samples were collected at a national retail pharmacy. Samples from San Diego County were collected as part of community testing organized by San Diego County. All age groups were represented, with 20-29 years old being the largest group (20.4% of samples) and 80-89+ years old the smallest age group (1.5% of samples) (**Table S1**). The individuals tested included multiple self-reported race categories (**Table S1**). We observed that the Omicron variant quickly grew to explain >99% of cases as of the week of January 17 (**Figure 1A**, **Table S2)**. Delta and Omicron variants therefore co-circulated (each representing >1% of infections) from 6 December 2021 to 23 January 2022, represented by 16,386 sequences in our dataset (**Figure 1A, Table S2**). During that time, the overall number of cases in the United States remained high, above 150,000 new cases per day and above a 7-day case rate of 250 per 100,000 individuals (CDC, 2020). The possibility of a co-infection by two distinct variants was therefore also high during this period.
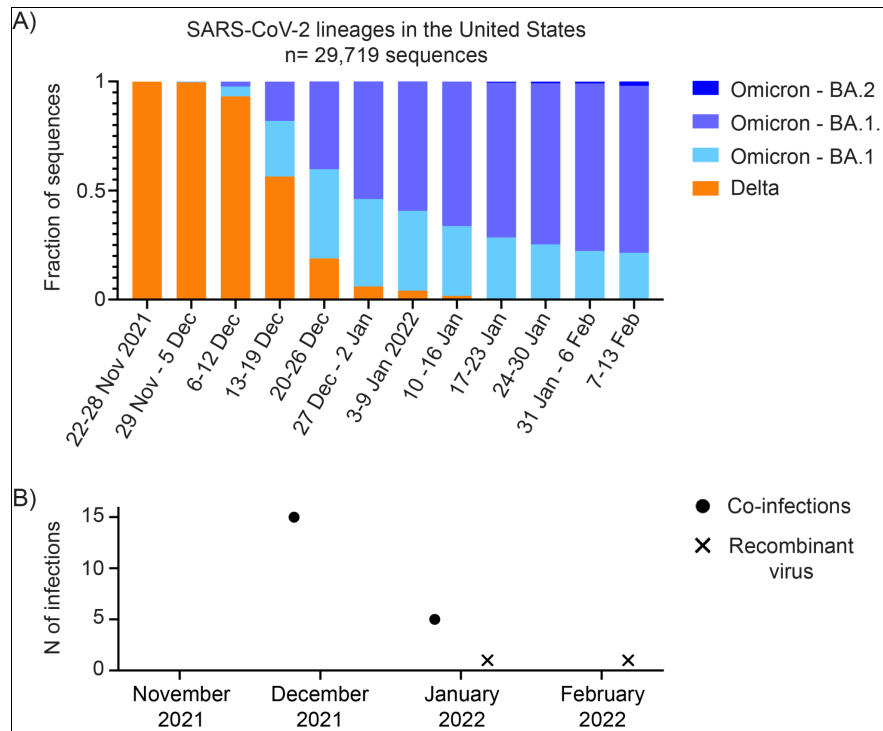
Figure 1: Co-circulation of Delta and Omicron variants in the United States. (A) Fraction of lineages sequenced per week in the United States. Delta includes B.1.617.2 and all lineages starting with AY. Detailed numbers for the most common AY lineages are in **Table S2**. Delta: orange. Omicron: light blue (BA.1), blue (BA.1.1), navy blue (BA.2). (B) Number of co-infections and infections from a recombinant virus per month. Co-infections are represented by a round symbol and infections from a recombinant virus by an X symbol.

Co-infection with Delta and Omicron variants

When a person is infected by two distinct variants/viruses, multiple copies of the full genome of each variant are present in the sample. A fraction (x%) of the total extracted SARS-CoV-2 RNA will come from variant A, and the remaining fraction ($100-x$%) of the RNA will come from variant B. Sequencing at a high enough coverage will lead to calling all mutations of both variant A and variant B, but each mutation will only be supported by a fraction of the reads overlapping the given position; the mutations specific to variant A should be called with ~x% of the reads overlapping the position, whereas the mutations specific to variant B should be called with ($100-x$)% **(Figure 2A)**. In order to identify this co-infection signature, we selected a list of mutations specific to the Delta variant and a list of mutations specific to the Omicron variant (**Table S3**). We

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assessed the fraction of samples having a call (not an "N") at positions of the selected mutations in all our samples between November 2021 and February 2022 (**Table S3**). All mutations selected had a call in >95% of the samples.

With a threshold median alternate allele fraction set at 0.85, we identified 21 samples that were very likely to be co-infected by Delta and Omicron variants. For 13 samples, we validated the results by re-sequencing them, or with an orthogonal genotyping assay (see Methods). The results replicated in 12 of the 13 samples (eight samples were unable to be re-tested). After removing the sample that did not replicate, this resulted in 20 positive samples that were co-infected by Delta and Omicron variants (**Figure 2B, Figure S1, Table 1**). A detailed list of the allele depths for each mutation for all of these samples is in **Table S4**. Of these 20, two have already been reported in a separate study (currently under review). The co-infections were enriched in samples collected in December 2021 when the fractions of Delta and Omicron were more similar (**Figure 1B**). However, three samples with co-infections were collected in January 2022, when Delta infections represented only 1.7% of the positive samples (**Figure 1A**, **Table S2**). Overall, we estimate that on average ~1 in 1,000 (20 / 16,386; 95% CI: 1/526 to 1/1,429, assuming a binomial distribution) positive samples between 6 December 2021 and 23 January 2022 had a co-infection.

Given how quickly Omicron displaced Delta in our dataset, but also throughout the world, we hypothesized that in cases of co-infections we would see on average more Omicron virions compared to Delta. Using the fraction of sequencing reads that mapped to mutations in either Delta or Omicron as a proxy, the fraction of Delta and Omicron virions in a given sample appeared similar (between 40 and 60%) in 9 out of 20 co-infections (**Table S5**). The Delta variant was higher than the Omicron variant in six co-infection samples (HMIX3, HMIX7, HMIX12, HMIX13, HMIX16 and HMIX18), while the Omicron variant was higher than the Delta variant in the remaining five (HMIX1, HMIX4, HMIX8, HMIX10, and HMIX17) (**Figure 2B, Figure S1, Table S5**). These results did not support our hypothesis that the Omicron variant would outcompete the Delta variant when in the same host. Our analysis is limited by the fact that we do not

have information whether the exposure and seed infection by the two distinct variants happened at the same time, or if they followed each other, which would have an impact on the relative viral load.

Table 1: Samples with co-infections and Delta-Omicron infections

| Name	Collection week	State	COVID Cq	Unique SARS-CoV-2 reads	Median AAF	Lineage	Clade	Replication method	Dominant variant	Breakpoint
HMIX1	Dec 2021	CA	18.1	480,979	0.71	None	21K (Omicron)	Re-extraction and re-sequencing	Omicron	Not Applicable
HMIX2	Dec 2021	NJ	16.6	625,507	0.53	None	21M (Omicron)	Re-extraction and re-sequencing	balanced	Not Applicable
HMIX3	Dec 2021	TX	21.0	10,131	0.47	None	21J (Delta)	Not available	Delta	Not Applicable
HMIX4	Dec 2021	CA	19.5	826,167	0.61	None	21K (Omicron)	Re-extraction and re-sequencing	Omicron	Not Applicable
HMIX5	Dec 2021	PA	19.2	1,164,229	0.54	None	21M (Omicron)	Re-extraction and re-sequencing	balanced	Not Applicable
HMIX6	Dec 2021	CA	17.4	1,998,992	0.51	None	21M (Omicron)	Re-extraction and re-sequencing	balanced	Not Applicable
HMIX7	Dec 2021	PA	19.3	320,813	0.43	B.1.617.2	21J (Delta)	Not available	Delta	Not Applicable
HMIX8	Dec 2021	CA	24.1	31,419	0.77	BA.1	21K (Omicron)	Not available	Omicron	Not Applicable
HMIX9	Dec 2021	FL	21.7	286,105	0.50	None	21K (Omicron)	Not available	balanced	Not Applicable
HMIX10	Dec 2021	CA	24.4	20,698	0.55	None	21K (Omicron)	Re-extraction and re-sequencing	Omicron	Not Applicable
HMIX11	Dec 2021	FL	20.4	193,976	0.54	None	21K (Omicron)	Re-extraction and re-sequencing	balanced	Not Applicable
HMIX12	Dec 2021	GA	21.1	359,378	0.83	AY.25	21J (Delta)	Re-extraction and re-sequencing	Delta	Not Applicable
HMIX13	Dec 2021	MI	21.1	668,533	0.51	B.1.617.2	21J (Delta)	Re-extraction and re-sequencing	Delta	Not Applicable
HMIX14	Dec 2021	CA	17.5	2,540,144	0.50	None	21M (Omicron)	Re-extraction and re-sequencing	balanced	Not Applicable
HMIX15	Dec 2021	TX	22.9	41,983	0.51	None	21K (Omicron)	Re-extraction and re-sequencing	balanced	Not Applicable
HMIX16	Jan 2022	FL	18.2	103,983	0.51	None	21J (Delta)	Not available	Delta	Not Applicable
HMIX17	Jan 2022	FL	21.8	354,336	0.81	BA.1.1	21K (Omicron)	Re-extraction and re-sequencing	Omicron	Not Applicable
HMIX18	Jan 2022	IN	17.4	3,930,358	0.44	None	21M (Omicron)	Not available	Delta	Not Applicable
HMIX19	Jan 2022	OK	19.9	1,121,368	0.54	None	21M (Omicron)	Not available	balanced	Not Applicable
HMIX20	Jan 2022	GA	19.7	692,308	0.49	None	21M (Omicron)	Not available	balanced	Not Applicable
RECOMB1	Jan 2022	MA	22.5	8,639	1.00	None	21K (Omicron)	Re-extraction and genotyping	Not Applicable	22,204 to 22,578
RECOMB2	Feb 2022	MA	20.5	240,742	1.00	None	21K (Omicron)	Not available	Not Applicable	19,220 to 21,618
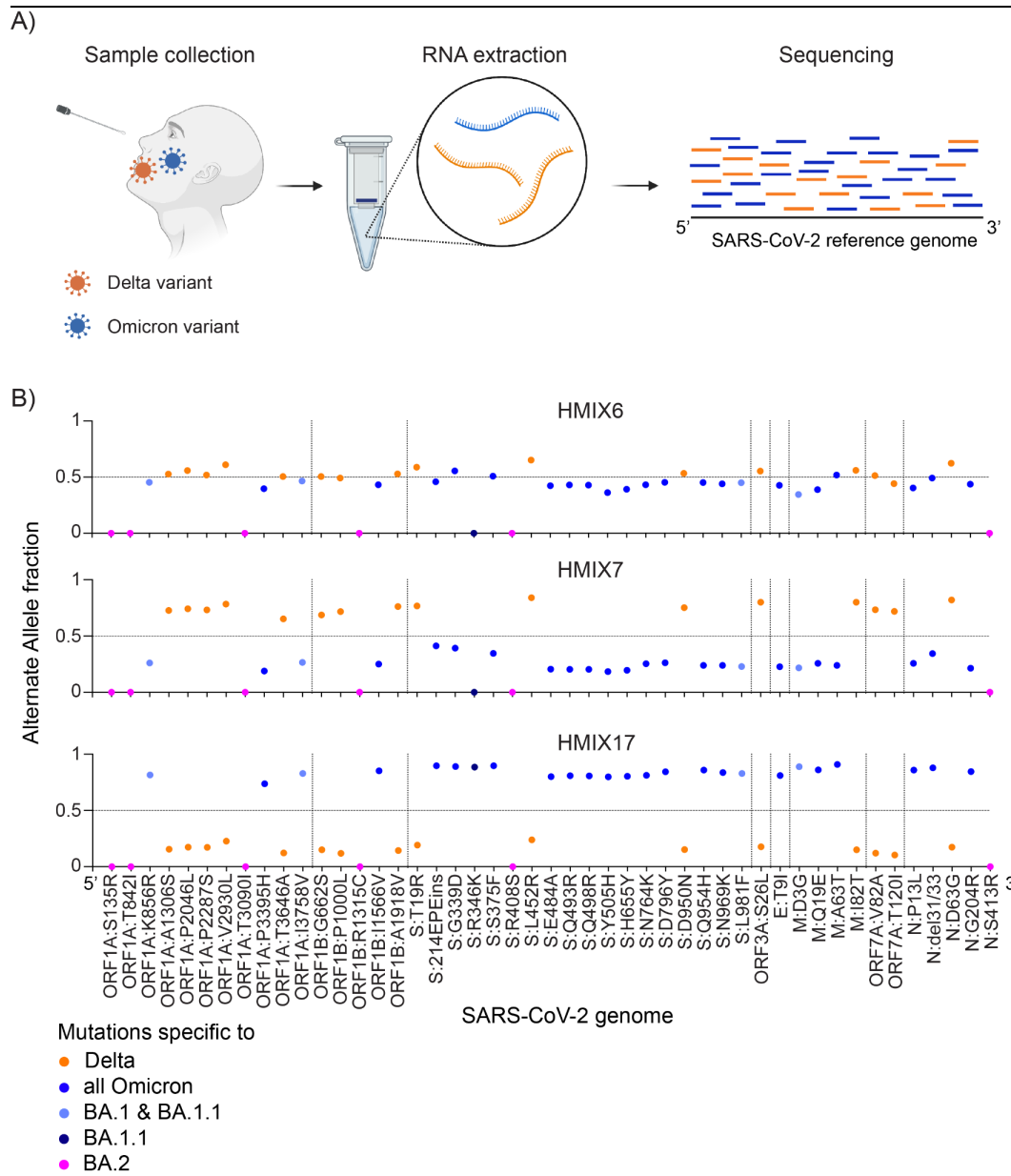
Figure 2: Co-infection with Delta and Omicron variants. (A) Schematic of a co-infection and the impact on the sequencing output. (B). Three example graphs representing the Alternate Allele Fraction for each mutation. Forty-seven mutations are plotted in order of their position on the SARS-CoV-2 genome from 5´ to 3´. Genes are separated by dashed vertical lines. Sixteen mutations specific to Delta are represented in orange. Twenty mutations specific to Omicron and shared by all its sub-lineages are represented in blue. Four mutations specific to BA.1 (and BA.1.1) are in light blue. The defining BA.1.1 mutation is in dark blue and 6 mutations specific to BA.2 are in magenta. The graphs for all 20 co-infections are in **Figure S1**.

Within-host recombination of Delta and Omicron genomes

We hypothesized that a subset of host cells in a co-infection would inevitably contain both variants, and therefore have the potential to generate recombinants. If these recombinants were replication competent, then we would detect them in sequencing output, manifesting as a change in allele fraction of defining mutations near the recombination breakpoint.

Indeed, we find that HMIX16 (**Figure 3A**) exhibits precisely this characteristic. Alternative allele fractions for Delta mutations hover around 0.80 near the 5´ end of the genome, but drop to around 0.50 near the beginning of the S gene and remain at this level until the 3´ end of the genome. This profile suggests the presence of a Delta-Omicron recombinant with a breakpoint preceding the S:214EPEins. We thus examined the read-pairs sequenced from HMIX16 that spanned mutations unique for Delta and Omicron upstream of S:214EPEins. Of the 21 read pairs that satisfied this criteria, we found 4 read-pairs that supported a Delta-Omicron recombinant, 7 read-pairs that supported Delta only, and 10 that supported Omicron only (**Figure 3B**). The read pairs that supported a Delta-Omicron recombinant comprise the S:G142D and S:156/157del mutations of Delta on the 5´ end, and the S:212del of Omicron on the 3´ end. One of these read pairs was long enough to also have the S:214EPEins.

The existence of these three unique mutation profiles presents compelling evidence that a recombinant virus was generated during co-infection with a breakpoint region of 157 base pairs between positions 22,036 and position 22,193. This recombinant replicated sufficiently to reach copy numbers that were detected by sequencing. As this sequencing data only provides a snapshot of the infection, it is not clear what within-host evolutionary dynamics led to the three populations, nor how they subsequently evolved post sample collection.
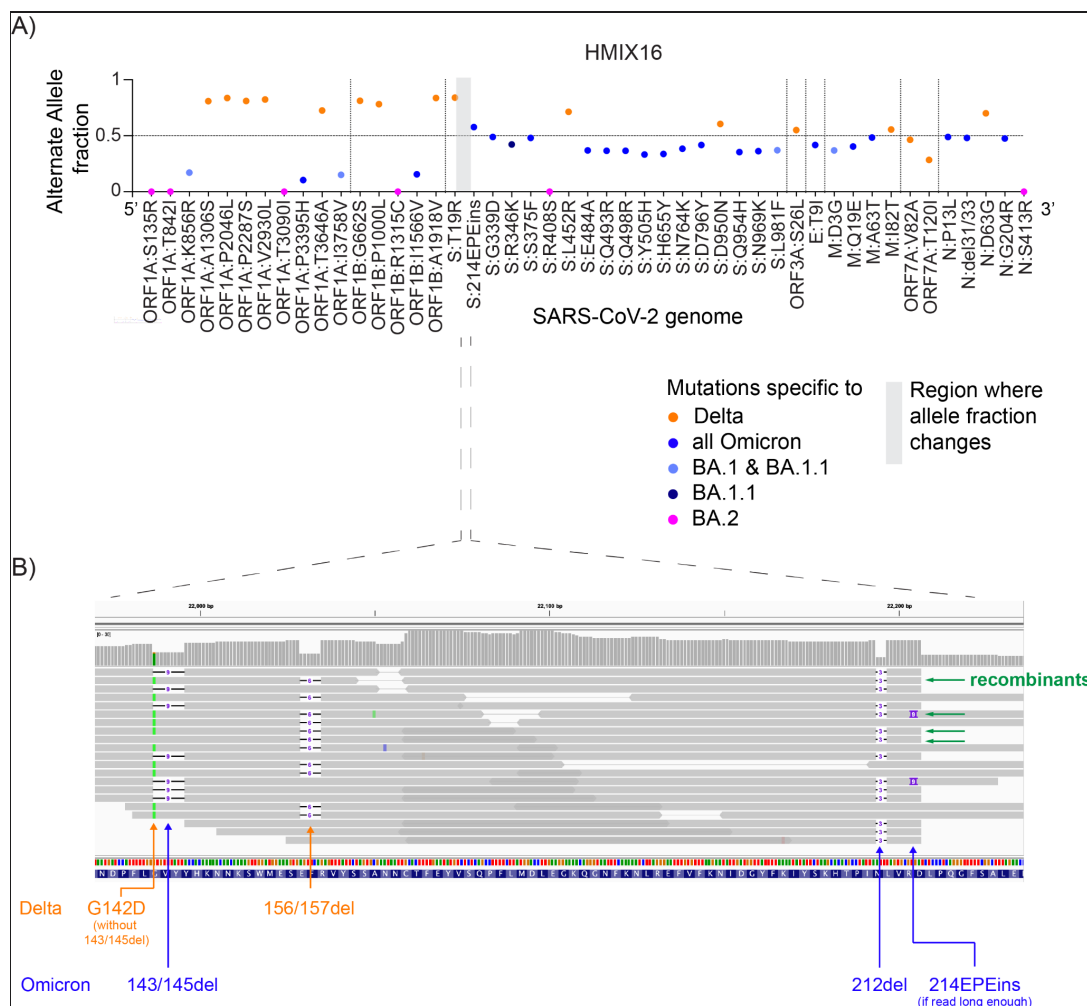
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Figure 3. Evidence of Delta, Omicron, and recombinant read pairs in HMIX16. (A) Graph representing the Alternate Allele Fraction for each mutation of sample HMIX16. Forty-seven mutations are plotted in order of their position on the SARS-CoV-2 genome from 5′ to 3′. Genes are separated by dashed vertical lines. Sixteen mutations specific to Delta are represented in orange. Twenty mutations specific to Omicron and shared by all its sub-lineages are represented in blue. Four mutations specific to BA.1 (and BA.1.1) are in light blue. One BA.1.1 mutation is in dark blue and 6 mutations specific to BA.2 are in magenta. The gray box represents the region where the alternate allele fraction changes. (B) An IGV (Robinson et al., 2011) view of the alignments for HMIX16, subsampled to only include read pairs where the first in pair covers the S:156/157del position, and the second in pair covers the S:212del position. Read pairs representing three mutation profiles are present: (i) supporting Delta mutations only (7 read pairs), (ii) supporting Omicron mutations only (10 read pairs), and (iii) supporting a Delta/Omicron recombinant (4 read pairs) marked with a green arrow. Mutations specific to Delta are represented in orange. Mutations specific to Omicron are represented in blue. Read pairs that do not span these mutations are not shown.

Infection with Delta-Omicron recombinants

Having established that co-infections occur and can generate replication competent virus, we then looked for samples that are composed entirely of recombinant virus. These could be infections that were seeded by a virus generated by the recombination of two distinct variants, or co-infections where recombination occurs during infection and the recombinant later comes to dominate the infection. In either case, we expect that all mutations called would be supported by ~100% of the reads because the sample collected is based on multiple copies of the same variant, rather than a mixture of two. Moreover, we expect only one breakpoint because (i) recombination is not that frequent, (ii) the viral genome is small and (iii) the period of analysis is only a few weeks after the first detection of co-infections. Therefore, all mutations identified on the 5´-end of the breakpoint should be characteristic of one variant (e.g., variant A), and all mutations on the 3´-end of the breakpoint should be characteristic of the other variant (e.g., variant B) (**Figure 4A**). We identified seven samples that had Delta-specific ORF1A:A1306S at the 5´-end of the genome, and Omicron-specific N:P13L at the 3´-end. One sample had Omicron-specific ORF1A:P3395H at the 5´-end and Delta-specific N:D63G at the 3´-end. Further analysis of these eight genomes showed that only two genomes, RECOMB1 and RECOMB2, had multiple consecutive Delta mutations at the 5´-end while the 3´-end of the genome had all of the Omicron mutations but none of the Delta mutations (**Figure 4B**). Four of the six other genomes had all (5´ to 3´ of the genome) Omicron-specific mutations and the additional Delta ORF1A:A1306S, which was probably acquired independently. The remaining genomes had all of the Delta-specific mutations with the additional Omicron N:P13L for one, and Omicron ORF1A:P3395H mutation for the other. These were probably also acquired independently.
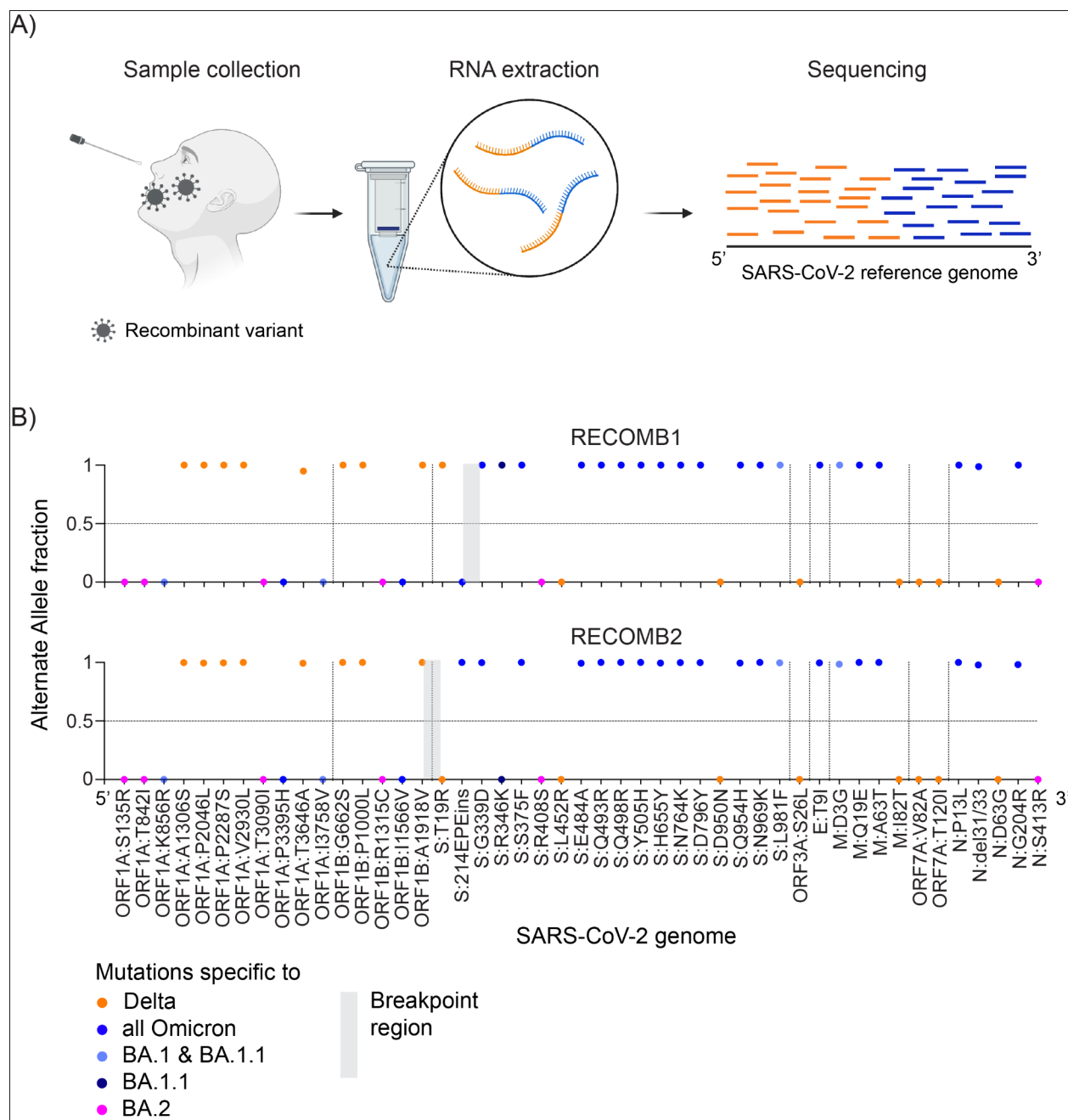
Figure 4: Infection with Delta-Omicron recombinants. (A) Schematic of the impact of an infection from a virus resulting from the recombination of Delta and Omicron on the sequencing output. (B) Graphs representing the Alternate Allele Fraction for each mutation. Forty-seven mutations are plotted in order of their position on the SARS-CoV-2 genome from 5′ to 3′. Genes are separated by dashed vertical lines. Sixteen mutations specific to Delta are represented in orange. Twenty mutations specific to Omicron and shared by all its sub-lineages are represented in blue. Four mutations specific to BA.1 (and BA.1.1) are in light blue. One BA.1.1 mutation is in dark blue and 6 mutations specific to BA.2 are in magenta. The gray box represents the homologous region where the breakpoint of the recombination is.

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Given that amplicon primer-based artifacts, in conjunction with laboratory contamination, have previously led to spurious signatures of recombination (Kreier, 2022), we were careful to check the quality of the two possible recombinant viruses. First, the library preparation method used by Helix's viral sequencing protocol is hybridization capture, not amplicon. Hybrid capture is less susceptible to artifacts due to mutations at primer sites, which has been a recurring issue with possible recombinant viruses observed in GISAID (Sanderson and Barrett, 2021). We have also seen far less drop-out in sequences generated via hybrid capture compared to amplicon in our sequencing data (**Figure S2**). Second, the Cq values for these two infections were low $Cq_{(RECOMB1)}$ = 22.5 and $Cq_{(RECOMB2)}$ = 20.5. Third, the sequencing output for these two samples passed our quality control criteria. RECOMB1 and RECOMB2 had sufficient yield and nearly 100% complete sequences, respectively: 8,639 and 240,742 unique reads that aligned to the SARS-CoV-2 genome (NC_045512.2); and 439 (1.5%) and 8 (0.03%) Ns. They also both had a median alternate allele fraction of 1 indicating that the majority of mutations were supported by 100% of the reads. Fourth, we performed a manual review of the alignments using IGV to make sure the reads supporting the mutations were of high quality. Lastly, we were able to validate our results for RECOMB1 by running a genotyping assay looking at Delta: C21618G (S:T19R at the protein level) and Omicron: G8393A, T13195C, C23202A (S:T547K at the protein level). The results showed the presence of Delta C21618G and Omicron C23202A in the sample, but the absence of Omicron: G8393A and T13195C. These confirm that the 5´-end of the genome was from Delta and the 3´-end from Omicron. Together, these experiments provide evidence that the two independent infections were caused by viruses resulting from the recombination of Delta and Omicron.

The sequences of the two recombinant viruses differ slightly. The breakpoint region of RECOMB1 is 374 bases between position 22,204 and position 22,578, while the breakpoint region of RECOMB2 is 2,398 bases between position 19,220 and position 21,618 (**Figure 4B**). Of interest, there is a private mutation T19404C in RECOMB2

inside the breakpoint region. RECOMB1 is a recombination between Delta and BA.1.1, while RECOMB2 is a recombination between Delta and BA.1. The full list of mutations including the presence of unlabeled mutations (not shared by a large fraction of genomes in the same lineage) are in **Table S6** (RECOMB1) and **Table S7** (RECOMB2) in a VCF-like format. These two samples were both collected in Massachusetts, but the difference in sequence suggests they are unrelated. Overall, infections from a recombinant Delta-Omicron virus remain rare: 2 out of 10,742 sequences between the weeks of January 10 and February 13, 2022.

Discussion

In this study we identified 20 cases of co-infection with the Delta and Omicron variants, and 2 cases infected by a virus resulting from the recombination of Delta and Omicron. While contamination could lead to the same output as a co-infection, several pieces of evidence discount contamination: (i) the fraction of reads supporting each variant was high in all cases (at least 15%); (ii) re-sequencing or an orthogonal test of these samples led to the same results for 12 samples and we were not able to perform a validation experiment for the remaining 8; (iii) samples that showed a co-infection were collected and processed on different days, and other samples sequenced on the same plates did not show co-infection; (iv) the collection dates of the co-infections and the infections by a recombinant virus followed a logical timeline; (v) in one of these co-infections, we found evidence of recombinant virus at a low but detectable frequency, consistent with template-switching during viral replication in a cell infected with two variants.

In the case of the two recombinants, we again think our data supports true chimeric sequences rather than being caused by technical artifacts, which are usually caused by the set of primers used (Kreier, 2022). First, we were able to replicate the result with an orthogonal genotyping assay for one of the sequences. Second, our sequencing protocol is based on hybrid capture and is less prone to PCR artifacts (**Figure S2**). Third, the chimeric exhibited breakpoint regions on the 5´ end of the spike protein as

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well as within the spike protein. These were not events limited to the spike protein, which is where most primer artifacts have been detected.

Our study demonstrates the existence of co-infections, the presence of a recombinant population in at least one of these co-infections, and the existence of two infections consisting almost entirely of multiple copies of a recombinant virus. However, the mechanism by which a recombinant virus comes to dominate an infection remains somewhat of a puzzle. One possibility is that the two infections that contain only recombinant virus were themselves seeded by a recombinant virus. This implies that in their respective ancestral co-infections, the two recombinant viruses each rose to a high enough fraction to be transmitted during an exposure and were able to establish an infection in a new host. Yet, despite transmitting to a new host at least once, the transmission chain was not sustained; we have not observed any more of these recombinants in our sequencing data. The other possibility is that these two infections began as co-infections and that the recombinant viral population then completely outcompeted the Delta and Omicron populations within the host. Yet, it seems unlikely that Delta and Omicron can be completely cleared from a host, while leaving the recombinant virus population intact. Either way, in both scenarios, the recombinant did not appear to have an increased ability to transmit between hosts compared to co-circulating Omicron variants. There are parallels here with HIV-1, where chronic infection and host immune response leads to extensive within-host diversity of the virus, but the genotypes of the virus that ultimately seed new infections are from a much narrower set of viral types (Joseph et al., 2015; Sagar et al., 2009).

With more diversity in SARS-CoV-2 and more time since the appearance of SARS-CoV-2, it will now be possible to track recombinations and have a better understanding of the rate of recombination. One way to look for these recombinants is the strategy we used. Another way is to carefully review every instance where a sample has good sequencing metrics but methods like Nextclade (Hadfield et al., 2018) or Pangolearn (Rambaut et al., 2020) have difficulty attributing a clade or a lineage to the sequence. Specialized methods have also been developed to detect recombination in

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viruses (Martin et al., 2015; Samson et al., 2021; Varabyou et al., 2021). Studies looking at the recombination rate of SARS-CoV-2 will then be able to compare it to other unsegmented positive-strand RNA viruses, as well as other viruses (Simon-Loriere and Holmes, 2011) in order to better understand the virus and anticipate what new variants or combination of mutations may arise.

Methods

Samples

This paper is based on the study of SARS-CoV-2 found and collected from individuals in the United States. The detailed demographics about these individuals can be found in **Table S1** of this paper.

The Helix data analyzed and presented here were obtained through IRB protocol WIRB#20203438, which grants a waiver of consent for a limited dataset for the purposes of public health under section 164.512(b) of the Privacy Rule (45 CFR § 164.512(b)). All samples were de-identified before receipt by the study investigators.

Helix COVID-19 test data and sample selection

All viral samples in this investigation were collected by Helix through its diagnostic testing laboratory. The Helix COVID-19 test is run on specimens collected across the US, and results are obtained as part of our standard test processing workflow using specimens from anterior nares swabs. The Helix COVID-19 Test is based on the Thermo Fisher TaqPath COVID-19 Flu A, Flu B Combo Kit, which targets three respiratory pathogens (SARS-CoV-2, Influenza A, and Influenza B). Test results from positive cases, together with a limited amount of metadata (including sample collection date, state, and qRT-PCR Cq values for all targets), were used to build the research database used here.

SARS-CoV-2 sequencing and consensus sequence generation

Sequencing was performed by Helix, as part of the SARS-CoV-2 genomic surveillance program in partnership with the Centers for Disease Control and Prevention (CDC). In the Helix workflow, RNA is extracted from 400 µl of patient anterior nares sample using the MagMAX Viral/Pathogen kit (ThermoScientific). All samples are subjected to total RNA library preparation using the Rapid RNA Library Kit Instructions (Swift Biosciences). SARS-CoV-2 genome capture is accomplished using hybridization kit

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xGen COVID-19 Capture Panel (Integrated DNA Technologies). Samples were sequenced using the NovaSeq 6000 Sequencing system S1 flow cell, which included the NovaSeq 6000 Sequencing System S1 Reagent Kit v1.5 (300 cycles).

Bioinformatic processing of this sequencing output is as follows. The flow cell output is demultiplexed with bcl2fastq (Illumina) into per-sample FASTQ sequences that are then run through the Helix klados-fastagenerator pipeline to produce a sequence FASTA file. First, reads are aligned to a reference comprising the SARS-CoV-2 genome (NCBI accession NC_045512.2) and the human transcriptome (GENCODE v37) using BWA-MEM. Reads are then marked for duplicates before proceeding to variant calling using the Haplotyper algorithm (Sentieon, Inc). Finally, the per-base coverage from the alignment file (BAM) and per-variant allele depths from the variant call format (VCF) file are used to build a consensus sequence according to the following criteria: coverage from at least 5 unique reads is required with at least 80% of the reads supporting the allele. Otherwise, that base is considered uncertain, and an N is reported.

Alternate allele fraction is the number of reads supporting an alternate allele (i.e. a mutation) divided by the total number of reads covering the position. The median alternate allele fraction is calculated as the median value of alternate allele fractions at sites where at least 15% of the reads support a mutation.

Viral lineage designation

Viral sequences were assigned a Pango lineage (Rambaut et al., 2020) using pangoLEARN (https://github.com/cov-lineages/pangoLEARN). For this analysis, pangoLEARN version 2022-02-02 with Pangolin software version 3.1.11 was used. We sequenced and were able to attribute a lineage to 29,719 sequences from samples collected between November 22, 2021 and February 13, 2022 for genomic surveillance purposes.

Genotyping

The detailed genotyping method as well as the validation of the method used in this study are previously described (Lai et al., 2022). The four specific markers used were:

- Delta: C21618G
- Omicron: G8393A
- Omicron: T13195C
- Omicron: C23202A

Relative fraction of each variant in co-infections

Number of RNA copies and coverage does vary across the SARS-CoV-2 genome. The density of mutations specific to Delta or Omicron also varies. There are many more Omicron-specific mutations in the spike protein. To try to minimize some biases, we took 4 Delta-specific mutations and 4 Omicron-specific mutations spread across SARS-CoV-2 genome to calculate the mean Delta-allele fraction and the mean Omicron-allele fraction in each sample. The 4 Delta-specific mutations are: ORF1A:P2046L, ORF1B:P1000L, S:T19R, M:I82T. The 4 Omicron-specific mutations are: ORF1A:P3395H, ORF1B:I1566V, S:N969K, M:A63T.

The results are in **Table S5**. We considered Delta to be the dominant variant if the Delta fraction was above 60% and the Omicron fraction was below 40%. We considered Omicron to be the dominant variant if the Omicron fraction was above 60% and the Delta fraction was below 40%. Other samples were considered balanced.

Data availability

All samples with a qc_status of pass were uploaded on GISAID.

GISAID identifiers for RECOMB1: hCoV-19/USA/MA-CDC-STM-HZEBR92XC/2022, EPI_ISL_9088187

GISAID identifiers for for RECOMB2: hCoV-19/USA/MA-CDC-STM-SP94WR2RW/2022, EPI_ISL_10114799

SRA BioSample accessions:

HMIX16: SAMN26527328, bioproject: PRJNA804575

(https://www.ncbi.nlm.nih.gov/biosample/26527328)

RECOMB1: SAMN26527329, bioproject: PRJNA804575

(https://www.ncbi.nlm.nih.gov/biosample/26527329)

RECOMB2: SAMN26527330, bioproject: PRJNA804575

(https://www.ncbi.nlm.nih.gov/biosample/26527330)

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Declarations of interest

A.B., S.W., T.B., A.D.R., D.W., E.K., H.D., T.C., K.T., J.N., J.R., S.C., E.T.C., K.S.B., N.L.W., S.J., E.S., D.B., J.T.L., M.I., W.L., and S.L. are all employees of Helix.

Supplementary Information

2 Supplementary figures and 7 supplementary tables

Figure S1: Co-infection with Delta and Omicron variants, related to Figure 2

Figure S2: Comparison of sequencing output based on Amplicon vs. Hybrid capture

Table S1: Demographic information on the population where the samples were collected

Table S2: Counts and fraction of different lineages in the United States by week, related to Figure 1

Table S3: List of mutations used to identify and differentiate Delta and Omicron variants

Table S4: Reference and Alternate allele depth for each mutation for all HMIX (co-infections) and RECOMB (Delta Omicron recombinant) samples

Table S5: Fraction of Delta vs Omicron in each co-infection samples

Table S6: List of mutations identified in RECOMB1 sample

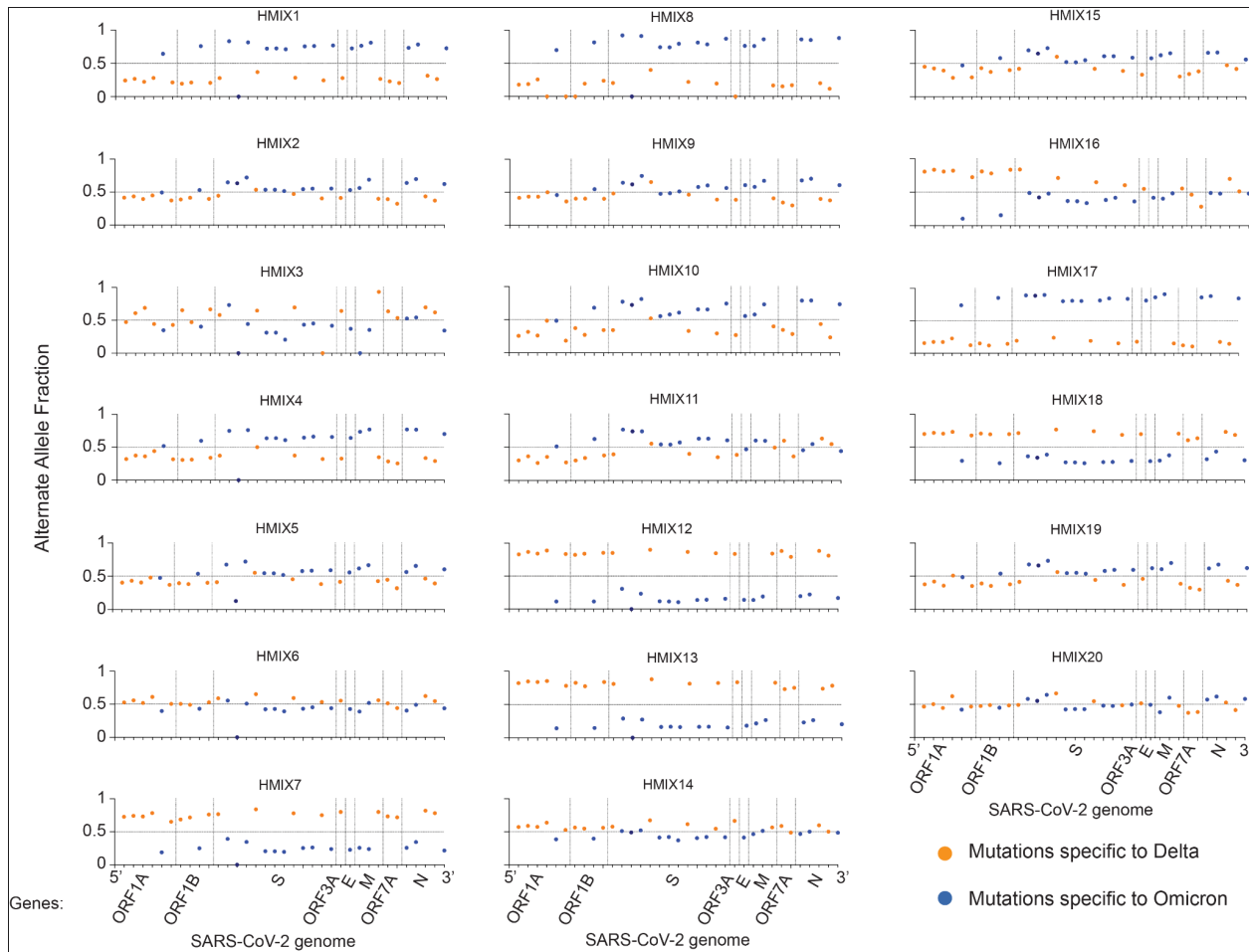**Table S7**: List of mutations identified in RECOMB2 sample

Figure S1: Co-infection with Delta and Omicron variants, related to Figure 2. Graphs representing the Alternate Allele Fraction for each mutation. 35 mutations are plotted in order of their position on the SARS-CoV-2 genome from 5´ to 3´. Genes are separated by dashed vertical lines. 18 mutations specific to Delta are represented in orange: ORF1A:A1306S, ORF1A:P2046L, ORF1A:P2287S, ORF1A:V2930L, ORF1A:T3646A, ORF1B:G662S, ORF1B:P1000L, ORF1B:1918V, S:T19R, S:L452R, S:P681R, S:D950N, ORF3A:S26L, M:I82T, ORF7A:V82A, ORF7A:T120I, N:D63G, N:R203M. 16 mutations specific to Omicron are represented in blue: ORF1A:P3395H, ORF1B:I1566V, S:G339D, S:S375F, S:E484A, S:Q498R, S:H655Y, S:N764K, S:D796Y, S:N969K, E:T9I, M:Q19E, M:A63T, N:P13L, N:DEL31-33, N:R203K. The S:R346K characteristic of Omicron BA.1.1 is in dark blue.
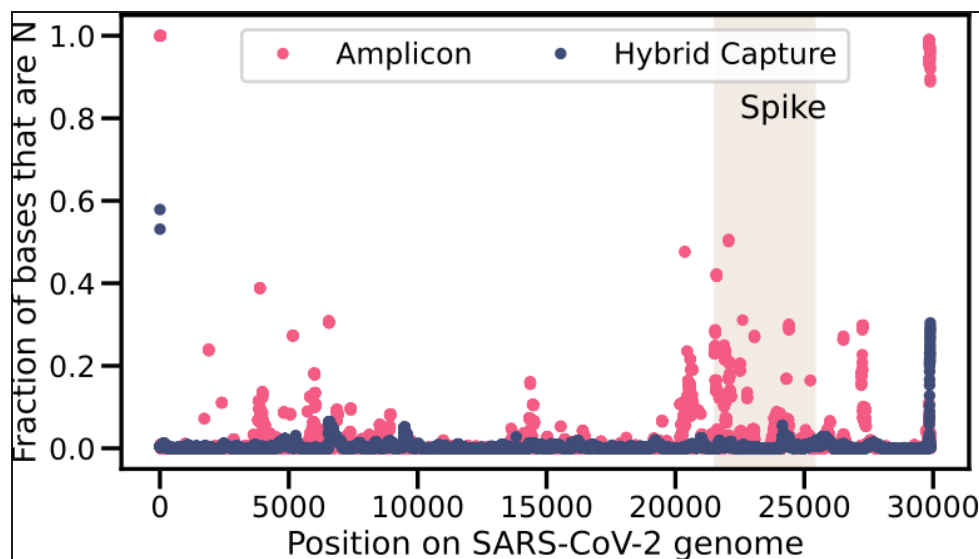
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Figure S2: Comparison of sequencing output based on Amplicon vs. Hybrid capture. Fraction of sequences with an N by position in the SARS-CoV-2 genome. The shaded rectangle corresponds to the region that encodes the S glycoprotein. Fraction based on 2500 positive samples sequenced via the amplicon method and 2500 positive samples sequenced via hybrid capture. The 2500 samples sequenced via hybrid capture include 174 that were designated as Omicron. None of the 2500 samples sequenced via the amplicon method were designated Omicron.

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