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Coronavirus Pandemic

Evaluation of sample pooling for gene sequencing of SARS-CoV-2: a simulation study

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) continues to pose a significant public health threat, requiring epidemiological and genomic surveillance. Next generation sequencing (NGS) is commonly utilized for monitoring viral evolution at a high cost. This study evaluated pooled sequencing as a cost-effective tool for monitoring virus variants.

Methodology: A simulation study was conducted to evaluate the efficacy of sample pooling for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) sequencing. In total, 72 original sets of raw data of gene sequencing with different genotypes were collected and combined to create 70 simulated samples based on five pooling strategies. A bioinformatics tool based on Freyja was utilized to analyze the variant composition of these 70 simulated pooled samples. The efficiency of recovering the correct genotypes of the original samples among different pooling strategies, result reports, and genotypes was evaluated with R software.

Results: The genetic composition of the pooled samples mostly recovered the genotype compositions of the original samples, with discrepancies between the top X results (where X is the number of original samples in the pool) and the complete results ($p < 0.05$). Variability in identification efficiency of genotypes were observed in the reports for the top X results ($p < 0.05$) across the five pooling strategies, but not in the reports of complete results ($p > 0.05$). Some original samples of low quality were not accurately identified.

Conclusions: Sample pooling coupled with streamlined genotyping offers a promising approach for cost-effective gene sequencing of SARS-CoV-2, which will aid in COVID-19 genomic surveillance.

Key words: SARS-CoV-2; sample pooling; gene sequencing; simulation study.

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Introduction

The impact of the coronavirus disease 2019 (COVID-19) pandemic on both human health and the social economy has been profound. The World Health Organization (WHO) conducted a study between 22 July to 18 August 2024 to estimate the impact of COVID-19 and reported that over 776 million confirmed cases and more than seven million deaths have been reported globally since the beginning of the pandemic [1]. The rapid mutation rate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to the emergence and global dissemination of new variants with distinct phenotypes in transmissibility, severity, and immune evasion [2]. Fourteen months after the World Health Organization (WHO) declared an end to the public health emergency of international concern regarding COVID-19, there were over 238,000 new cases reported across 91 (39%) countries, and about 4,400 new fatalities reported across 35 (15%) countries during the 28-day period (from July 22, to August 18, 2024), primarily attributed to the prevalence of the lineages of JN.1 and the variant KP.3.1.1 [1]. Ongoing genomic surveillance remains crucial for addressing the persistent threat of new variants and outbreaks due to the continuous evolution and spread of the SARS-CoV-2 virus [3]. In the post-pandemic era, the focus of genome sequencing has shifted from individual diagnosis and molecular epidemiology tracing; to the surveillance of population spread, and the genetic diversity and evolution of SARS-CoV-2 within communities. The viral genotypes and their compositions in populations can be used to track the emergence of new variants and genetic changes, which is of significant importance for early warning and risk assessment of COVID-19.

Next generation sequencing (NGS) is widely used to monitor viral evolution. Notably, the sequences of SARS-CoV-2 shared on GISAID (https://gisaid.org/) vary significantly across continents, with Europe contributing the most and Africa the least (even lower than Oceania). Cost and sequencing throughput are likely limitations affecting the use of genomic surveillance in tracking the possible risk of COVID-19. These factors can influence our understanding of the prevalence of variants of concern or interest (VOCs/VOIs). A high-throughput, low-cost sequencing method will offer notable advantages and be valuable for public health.

During the COVID-19 epidemic, the practice of pooling SARS-CoV-2 samples was utilized for population screening due to its recognized benefits [4–5]. Pooling samples increase the throughput of molecular testing and reduce costs [6], making them an economically advantageous option. However, the challenge lies in the results analysis of pooled sequencing, specifically in accurately estimating the composition of multiple SARS-CoV-2 lineages within samples containing mixed viral populations. A bioinformatics analysis method using Freyja has revolutionized the challenges of analyzing mixed sequencing data and has been applied in the sequencing of sewage (mixed) samples for SARS-CoV-2 [7], indicating the potential for population-based pooled sequencing. Pooled sequencing presents a cost-efficient approach for acquiring sufficient genomic data in areas with constrained sequencing capabilities and high demand, thereby enriching our understanding of viral evolutionary and transmission patterns.

This study aimed to assess the efficacy of lineage classification for SARS-CoV-2 in various simulated pooling samples. The objective was to evaluate the potential of pooled sequencing as a genomic surveillance tool for COVID-19.

Methodology

Original samples

The original raw data (in fastq.gz format) from gene sequencing procedures carried out using Illumina NGS platforms (Illumina, USA) were collected in our laboratory between 2020 and 2024. This dataset included samples from individuals and vaccine materials (CoronaVac, the Sinovac inactivated SARS-CoV-2 vaccine). A coverage exceeding 96% was the fundamental criterion for inclusion of effective sequences in our country; while a depth surpassing 1000× was determined by the average sequence depth acquired in our laboratory. It was observed that data with depths below this threshold were generally of low quality. Subsequently, samples meeting our criteria (minimum 96% coverage and depth exceeding 1000× for the entire genome of SARS-CoV-2) were identified as genotype-determined specimens. In addition, to test the impact of low-quality sequencing samples on the results, data from two low-quality sequencing samples were randomly included.

The study established various genotype groups at different hierarchical levels: 4 in the first-level classification including the groups of Original*, BA*, XBB*, and recombinants; six in the second-level classification by dividing the genotypes of BA*and XBB* into four subgroups (BA.1 + BA.5, BA.2, XBB.1.9*, and XBB* without XBB.1.9*), and 38 of subdivided subtypes in the third-level classification. A total of 72 samples representing all the available subtypes in our laboratory were included in the study (Supplementary Table 1). A total of 500,000 raw data reads were randomly extracted for each sample using the subsample tool of CLC Workbench 23.0 software (Qiagen, Dusseldorf, Germany) to create original samples.

Pooling strategy design

Potential interactions among genotypes of SARS-CoV-2 in real-world scenarios by integrating genotype classification and historical prevalence of the virus were assessed, and 5 distinct pooling strategies were developed. Five groups of mixtures were created to simulate sample pooling for gene sequencing using the Create Sequence tool within the CLC Workbench 23.0 software (Qiagen, Düsseldorf, Germany). The details of the simulated samples and mixtures are listed below and presented in Table 1.

1) A mixture of 5 groups was formed based on the second-level classification, excluding “recombinants”. One random sample from each group was selected and mixed equally, labeled as “mix.”

2) The samples were classified into 3 specific groups based on the first-level classification, excluding “recombinants”. A mixture of transition between 2 groups, comprised of movement from the original* stage to the BA* stage, and from the BA* stage to the XBB* stage. Random samples were selected from each of the 2 groups at varying ratios of 1:4, 3:2, and 4:1, respectively; and combined as a mixture labeled as “change.”

3) The samples were classified into three specific groups based on the first-level classification, excluding

"recombinants". Five random samples were selected from each group and combined, then labeled as "genotype-class I".

4) The samples were grouped into 8 categories based on prevalent lineages, with Delta\XBB.1.5\XBB.1.9.1\XBB.1.9.2\XBB.1.16\BA.5.2\BA.5.2.48&49\JN being the most common. Each group was combined to create one simulated sample labeled as "genotype-class II".

5) Three recombinants of XBF/XBL/XBC, along with 3 simulated recombinants of XBG/XBC/XDD; in total 6 recombinants based on the third-level classification; were identified and labeled as "recombinants".

Data analysis

The combined data from simulated samples underwent genotype and abundance analysis using the bioinformatics software module within the Pathogenic Microbial Analysis System (V1.0.6, MicroFuture, Beijing, China). Specifically, the analysis was conducted utilizing the SARS-CoV-2 Analysis Module for Environmental Samples of the software. This system is underpinned by the Freyja algorithm and makes use of the genotyping tool Nextclade (https://clades.nextstrain.org/).

The study assessed the lineage abundances, ranked genotype composition by abundance, and examined the genetic consistency between simulated pooled samples and the original samples. The consistency assessment was mainly based on the "genotype of original samples" (Table 1). This evaluation was conducted based on the top X results (X representing the number of original samples in the mixture) and the complete results.

The efficiency of recovering the correct genotypes of the original samples using different pooling strategies, result reports, and genotypes was evaluated with R software (http://www.R-project.org, version 4.2.1). The categorical variables were analyzed using either the Chi square test or Fisher's exact probability method (when there were expected frequencies below 5 in the 2×C table) to compare the differences in gene identification efficiency among different hybridization strategies and result reports. Statistical significance was defined as a p value < 0.05.

Results

General identification efficiency of pooled sequencing

This study included 72 original samples and 70 simulated pooled samples (details in Supplementary Table 2). Among these, 39 simulated samples precisely matched the original genotypic compositions (good), while 24 samples had one missing genotype (mediocre), and 7 samples had two missing genotypes (bad); as determined by the top X results. In contrast, 61 simulated samples exactly replicated the genetic compositions of the original samples, with 9 samples containing one missing genotype as determined by the complete results. There were no "bad" results observed in the entire output. The complete results demonstrated superior identification compared to the top X results across all pooling strategies. Among the various

Table 1. Pooling strategy for mixture as simulated samples.

Group	Classification	Pooling strategy for mixture	Counts of simulated samples
Mix	Second level	1 out of each 5 groups randomly (excluding recombinants group)	5
Change	First level	Original (1):BA*(4)	5
		Original (3):BA*(2)	5
		Original (4):BA*(1)	5
		BA*(1):XBB*(4)	5
		BA*(3):XBB*(2)	5
		BA*(4):XBB*(1)	5
Genotype-Class I	First level	5 out of the group of Original randomly	5
		5 out of the group of BA* randomly	5
		5 out of the group of XBB* randomly	5
Genotype-Class II	Third level	all the samples of Delta* group	1
		all the samples of XBB.1.5* group	1
		all the samples of XBB.1.9.1* group	1
		all the samples of XBB.1.9.2* group	1
		all the samples of XBB.1.16* group	1
		all the samples of JN* group	1
		all the samples of BA.5.2* group	1
		all the samples of BA.5.2.48/49* group	1
Recombinants	Third level	XBF	1
		XBL.3	1
		XBC.1.6.2, XBG#, Omicron (BA.2.76)*1 + Omicron (BA.5.2)*3	3
		XBC#, Omicron (BA.2) *1, + Delta (B.1.617.2*)*3	3
		XDD#, Omicron (EG.5.1.1) *1 + Omicron (JN*)*3	3
Total			70

*: including the lineage and its subtypes.

Table 2. Consistency of simulated pooling samples and original samples among various pooling strategies.

Variable	**Consistency**					
	N	**Identified (N)**	**Identified (%)**	**Unidentified (N)**	**Unidentified (%)**	**p value**
Result-rank						
Top X	70	39	55.71	31	44.29	< 0.001#
Complete	70	61	87.14	9	12.86	($\chi^2 = 16.94$)
Pooling strategy-top X						
Mix	5	0	0.00	5	100.00	0.003*
Change	30	15	50.00	15	50.00	
Genotype-class i	15	7	46.67	8	53.33	
Genotype-class ii	8	6	75.00	2	25.00	
Recombinant	12	11	91.67	1	8.33	
Pooling strategy-complete						
Mix	5	3	60.00	2	40.00	0.256*
Change	30	26	86.67	4	13.33	
Genotype-class i	15	13	86.67	2	13.33	
Genotype-class ii	8	7	87.50	1	12.50	
Recombinant	12	12	100.00	0	0.00	

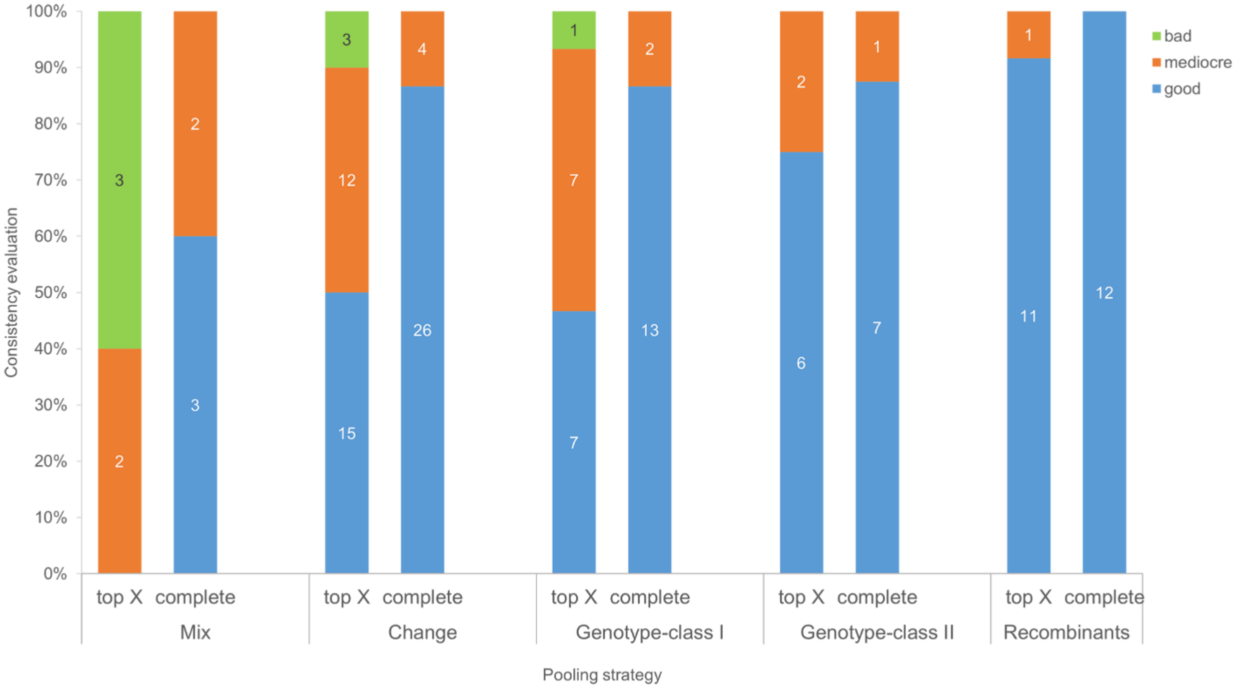
#, Pearson's Chi-squared test; *, Fisher's exact test.

pooling strategies, the "mix" group consisting of five original samples with completely different genotypes exhibited the poorest identification, while the "recombinants" group with one or two original samples displayed the highest level of identification. The blending of closely related genotypes within groups such as "genotype-class I" and "genotype-class II" yielded approximately average identification results, with over 80% distinguishable outcomes as indicated by the complete results, along with the remaining group of "change". The genetic compositions of the simulated pooling samples largely reflected the correct variant proportions of the original samples, with variability observed across different pooling strategies, as depicted in Figure 1.

Identification efficiency among different pooling strategies and result reports

By categorizing consistent results as identified and missing results as unidentified, the statistical analysis revealed significant differences in the top X and complete reports, with a Chi square value of 16.94 ($p < 0.001$). The complete results revealed a higher percentage of identified samples (87.14%) compared to the top X results (55.71%). While there was variability in the top X result reports among the five groups ($p =$

Figure 1. The identification of original genotypes by different pooling strategies.



The identification of original genotypes by different pooling strategies in the proportions of the original subtypes by the top X results and complete results, respectively. Gray color represents bad with two genotypes missing, orange represents mediocre with one genotype missing, and blue represents good with no genotype missing.

0.003, < 0.05), the complete reports showed no significant differences in the pooling strategies employed (p = 0.256, > 0.05). Analysis of the top X results indicated that the "mix" group did not accurately recover the genetic compositions of original samples, but successfully identified the majority of samples (91.67%) within the "recombinants" group. Overall, the identification accuracy improved in the complete results across all pooling strategies compared to the top X results (Table 2).

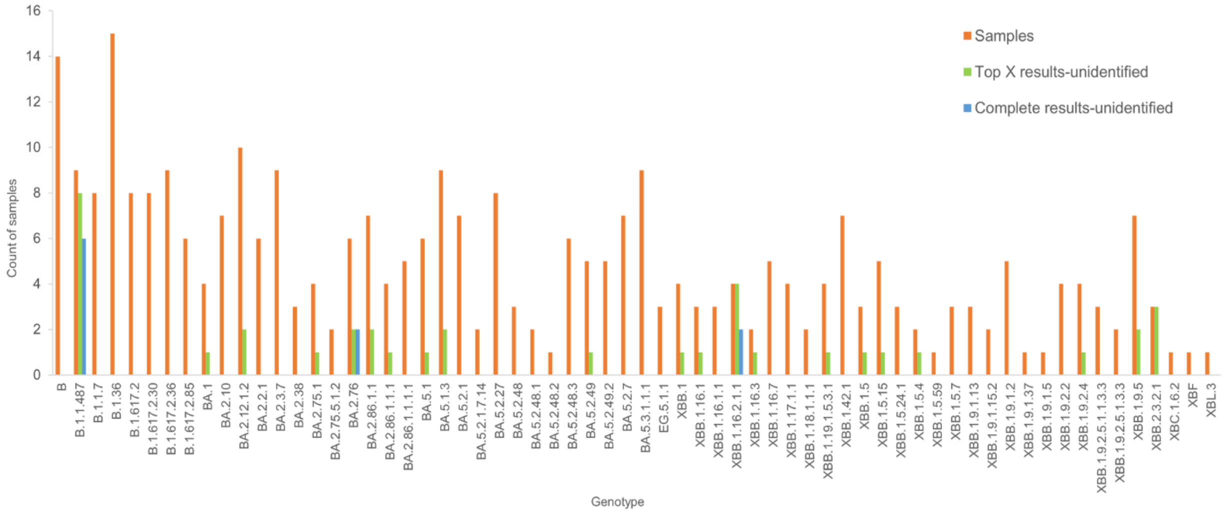
Identification efficiency among different genotypes

Regarding specific genotypes, the majority of original samples were accurately identified within mixed samples based on both the top X results and complete results. The complete results also successfully identified some previously unidentified genotypes from the top X results. However, a few original samples were not correctly identified in either the top X results or the complete results. Examples include B.1.1.48 (mistaken for B.1.551 or missing), BA.2.76 (not consistently detected in most simulated samples), and XBB.1.16.2.1.1 (identified as XBB.1.16.2 or XBB.1.16.2.1). This is illustrated in Figure 2. It is important to note that a majority of the unidentified pooled samples contained low-sequencing-quality original samples, as mentioned above.

Discussion

Continuous monitoring and tracking of the mutations and variants of SARS-CoV-2 is crucial for risk assessment and early epidemic warning; and the cost-effective pooled sequencing strategy may facilitate progress in this endeavor. Unlike qualitative pooling tests, in real time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR), pooled sequencing necessitates a more intricate analysis involving mutation definition, lineage identification, and quantitative assessment of each lineage. Existing tools for SARS-CoV-2 lineage classification, such as Phylogenetic Assignment of Named Global Outbreak LINeages (Pangolin) or Ultrafast Sample Placement on Existing Trees (UShER), are primarily designed for clinical samples dominated by a single variant [8,9]. In the case of pooled samples, the complexity has been addressed through the development of the Freyja approach. The bioinformatics analysis tool was utilized to estimate the relative frequencies of SARS-CoV-2 variants, employing a statistical model that incorporates a predefined set of genomic polymorphisms specific to these variants [7]. In other words, Freyja uses a “barcode” library of lineage-defining mutations to represent each lineage in the global phylogen for SARS-CoV-2, and then restores relative lineage abundance by solving the depth-weighted least absolute deviation regression problem. This approach has demonstrated efficacy in monitoring sewage samples across several countries [10,11]. In 2023, we successfully applied the Freyja tool in urban sewage monitoring, and obtained information of the genomic composition and abundance for SARS-CoV-2. In this study, the patient swab samples had higher viral loads and were less diluted compared to wastewater samples. The software based on Freyja effectively identified the correct variant proportions in the majority of simulated samples. In fact, this tool also provided a

Figure 2. The identification of original genotypes among different genotypes.



The numbers of unidentified samples of different original genotypes by the top X results and complete results, respectively in gray and blue.

comprehensive list of all mutations present which was useful for genomic monitoring (not shown in this study). Moreover, as an innovative bioinformatics analysis approach, Freyja has been incorporated into diverse software tools for pooled sequencing of SARS-CoV-2, and it is also compatible for self-analysis using R software. Nonetheless, there are notable considerations regarding the implementation of pooled sequencing in genomic surveillance that warrant further attention.

Not all simulated samples were accurately recovered, primarily due to original samples lacking definitive mutations and exhibiting lower recognition quality. For instance, the simulated samples harboring B.1.1.48 (assessed as low-quality by Nextclade) and BA.2.76 (with less than 96% coverage) led to 36.8% unidentified cases among the top X results and 80% among complete results. Therefore, ensuring the sequencing quality of original samples before pooling is essential, because samples of poor quality can compromise identification accuracy. High-quality nucleic acid, characterized by both high concentration and purity, plays a key role in achieving superior sequencing outcomes, yet ensuring this may be challenging due to various factors that come into play during the sequencing process. However, the assessment of sequencing quality in the absence of sequence testing for the original samples raises questions regarding the feasibility of using cycle threshold (Ct), DNA integrity number (DIN), or RNA integrity number (RIN) values as indicators alone. Further investigation is required to establish quality control measures prior to pooling. We are trying to assess the quality of the library construction process to derive evaluation parameters that are more pertinent to sequencing quality, thereby enhancing the accuracy of sample selection in pooled sequencing.

Additionally, observations indicated that samples with lower viral loads could be overlooked in the final pooled RT-qPCR result [12], emphasizing the importance of pooling samples with high and similar nucleic acid concentrations. In simulated experiments, each sample is mixed with an equal number of reads, but the situation may vary in actual detection. Drawing from our experience in routine sequencing, adjustments in the proportion of labeled samples can be made based on the concentration of nucleic acids or libraries to ensure balanced data acquisition from each sample. Therefore, volume adjustments in pooled sequencing can also be tailored according to the nucleic acid concentration of original samples to achieve a more uniform mixing, optimizing abundance balance, and reducing the likelihood of missed detection due to low data yield. When multiple original samples in a pooled sample share the same genotype, they become indistinguishable in pooled sequencing. Notably, adherence to the principle of approximately equal mixing may result in higher abundance, indicating a greater composite representation. Moving forward, the development of mathematical models could aid in mitigating such interference in pooled sequencing.

We also detected some unexpected variant sites. This may be either a misjudgment or the result of amplifying low-frequency variants in each sample due to an enrichment effect. This could serve as a meaningful early warning for exploring variants, warranting further investigation.

The software utilized for analyzing genomic composition ranked the abundance results, revealing differences between the top X results and the complete results. Upon simulating various pooling scenarios, statistical discrepancies were noted among the different pooling strategies, particularly when focusing on the top X results. Optimal consistency between the simulated samples and original samples was observed in the “recombinants” group, while the “mix” group showed the lowest level of consistency. These findings suggest that the complexity of the pooled samples significantly affects the accuracy of identification as well.

While the complete results demonstrated improved identification compared to the top X results, there were instances of inaccurate genotyping due to the presence of low-abundance mutation mixtures. The Freyja method incorporated a bootstrap technique to calculate standard errors for predicting variant compositions. However, determining the optimal cutoff value for genomic composition results that strike a balance between sensitivity and specificity remained a challenge. An additional constraint is that pooled sequencing can only determine the viral genotype compositions and abundance, necessitating individual identification when new variants emerge, similar to individual confirmation in pooling tests for RT-qPCR [13].

The study commenced by sporadically conducting gene sequencing on a pooled sample of routine tests, and successfully recovered most of the genotypes present in the individual samples (Supplementary Table S3). Due to the impracticality of pooling numerous samples into diverse groups, a simulated study was conducted to assess the feasibility of pooled sequencing. The pooling strategy designed based on historical data aligned well with the real world and recent data also confirmed this. The monitoring of genomic diversity in the population in Chengdu in 2023 showed distinct phases throughout the year. Initially, the prevalent strains were BA.5.2.48, which gradually declined by week 19. Subsequently, a combination of major stains XBB.1.5, XBB.1.9.1, and XBB.1.9.2 were observed between the 12th and the 27th week. From the 27th week onwards, XBB.1.9.2 emerged as the predominant strain. This was similar to the pooling strategy of "change". Since 2024, the dominant genotypes have consistently been the JN lineage and XDV recombinants. The patterns of genotype mixing resembled the strategic pooling of "genotype-class II", "recombinant", and "change". If the one-in-five mixed sampling was implemented in practice, it would result in a potential cost saving of around 80%, making it a highly cost-effective approach to monitoring.

Existing models for pooling tests in RT-qPCR [14,15] and wastewater-based epidemiological monitoring [16] offer valuable insights for implementing pooled sequencing for SARS-CoV-2. However, additional details are necessary for practical application, including the pooling procedure, pooling size, influencing factors, quality control of original samples, and optimization strategies of bioinformatics software. The rapid variation of the SARS-CoV-2 continually pose risks of new variants emerging and subsequent outbreaks. The presence of post-acute sequelae of SARS-CoV-2 (PASC) and incomplete understanding of the virus may cause more health hazards than common respiratory diseases such as influenza. Although the public attention towards COVID-19 has waned, it remains a virus requiring vigilance, as evidenced by the monthly updates from GISAID and reports from the WHO. Regular population surveillance remains an ongoing standard practice for the sake of public health. Timely awareness of virus variants and trends in their prevalence plays a constructive role in making prompt public health decisions, such as vaccine development, drug stockpiling, allocation of medical resources, and adjustments in prevention and control policies.

Pooled sequencing is recommended for population monitoring to approximate virus genotypes and their compositions, as the primary objectives. The approach aims to monitor virus variations, determine population prevalence, shift the focus from individuals to the overall population, and offers efficiency and cost advantages in this context. Nevertheless, for detailed genotypic analysis of SARS-CoV-2 or advanced research purposes, the traditional single sequencing methods remains indispensable.

Conclusions

This study utilized simulated mixed samples to assess the feasibility of pooled sequencing with analysis using the Freyja tool. The findings demonstrated the successful recovery of the gene composition of the original samples. Therefore, pooled sequencing presents itself as a promising tool that can enhance genomic surveillance efforts in combating COVID-19 in a cost-effective manner.

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Authors' contributions

Conceptualization: HC, LW; methodology and software analysis: XZ, validation: HC; formal analysis: XZ; experiments, resources, and data curation: XH, YZ, WX, DS, ZH, RL, WL; writing – original draft: HC, YC; writing – review and editing: XZ, LW; supervision: LW; funding acquisition: XZ, HC, YC. All authors have read and agreed to the published version of the manuscript.

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Annex - Supplementary Items

Supplementary Table 1. The definitions of genotype groups at various levels of the original samples.

First-level classification	Second-level classification	Third-level classification	Genotype of original samples
Original	Original	B	B
		B.1.1*	B.1.1.7
			B.1.1.487
		B.1.36	B.1.36
		Delta	B.1.617.2
			B.1.617.2.30
			B.1.617.2.36
			B.1.617.2.85
BA*	BA.1+BA.5	BA.1	BA.1
		BA.5.1	BA.5.1
		BA.5.1.3	BA.5.1.3
		BA.5.2.1*	BA.5.2.1
			BA.5.2.1.7.14
		BA.5.2.7	BA.5.2.7
		BA.5.2.27	BA.5.2.27
		BA.5.2.48*	BA.5.2.48
			BA.5.2.48.1
			BA.5.2.48.2
			BA.5.2.48.3
		BA.5.2.49*	BA.5.2.49
			BA.5.2.49.2
	BA.2	BA.5.3.1*	BA.5.3.1.1.1
		BA.2.2*	BA.2.2.1
		BA.2.3*	BA.2.3.7
		BA.2.10	BA.2.10
		BA.2.12*	BA.2.12.1.2
		BA.2.38	BA.2.38
		BA.2.75*	BA.2.75.1
			BA.2.75.5.1.2
		BA.2.76	BA.2.76
		JN*	BA.2.86.1.1
			BA.2.86.1.1.1
			BA.2.86.1.1.1.1
XBB*	XBB*(excluding XBB.1.9*)	XBB.1	XBB.1
		XBB.1.5*	XBB.1.5
			XBB.1.5.4
			XBB.1.5.7
			XBB.1.5.15
			XBB.1.5.24.1
			XBB.1.5.59
		XBB.1.16*	XBB.1.16.1
			XBB.1.16.1.1
			XBB.1.16.2.1.1
			XBB.1.16.3
			XBB.1.16.7
		XBB.1.17*	XBB.1.17.1.1
		XBB.1.18*	XBB.1.18.1.1.1
		XBB.1.19*	XBB.1.19.1.5.3.1
		XBB.1.42*	XBB.1.42.1
		XBB.2.3*	XBB.2.3.2.1
	XBB.1.9*	XBB.1.9.1*	XBB.1.9.1.2
			XBB.1.9.1.5
			XBB.1.9.1.13
			XBB.1.9.1.15.2
			XBB.1.9.1.37
		XBB.1.9.2*	XBB.1.9.2.2
			XBB.1.9.2.4
			XBB.1.9.2.5.1.3.3
			XBB.1.9.2.5.1.1.3.3
		XBB.1.9.5*	XBB.1.9.5
Recombinants	Recombinants	XBF	XBF
		XBL.3	XBL.3
		XBC.1	XBC.1.6.2
		XBG#	Omicron (BA.2.76) + Omicron (BA.5.2)
		XBC#	Omicron (BA.2) + Delta (B.1.617.2*)
		XDD#	Omicron (EG.5.1.1) +Omicron (JN*)

*, including the lineage and its subtypes; #, simulated samples.

Supplementary Table 2. The original genotypes and the recovered genotypes of 70 simulated samples.

No.	Name	Original genotypes					Name	Recovered genotypes													
1	M-1	B.1.1.7	BA.5.1	BA.2.10	XBB.1	JG.3	M-1	3490.46													
		B.1.1.7	BA.5.1	BA.2.10	XBB.1	XBB.1.9.2.5.1.3.3		summarized	[('Omicron',	0.7479918062126736),	('Alpha',	0.1921229949875294)]									
		√	BA.5.1.12	√	——	√		lineages	B.1.1.7	BA.2.10	BA.5	JG.3	BA.5.1.12	XBB.1.5	XBB.1.1	XU	XBB	EG.5	Q.7	EG.5.1.8	BA.5.3
		√	BA.5.1.12	√	XBB.1.1	√		abundances	0.16897169	0.12384649	0.10786812	0.10358644	0.09602485	0.07438334	0.06180715	0.05509775	0.05337512	0.03120199	0.0231513	0.02271817	0.0180824
								resid	15.07037287												
								coverage	99.84945301												
2	M-2	B	DY.3	BA.2.3.7	XBB.1.5	XBB.1.9.5	M-2	3693.13													
		B	BA.5.2.48.3	BA.2.3.7	XBB.1.5	XBB.1.9.5		summarized	[('Omicron',	0.7175736254249501),	('Other',	0.07696567717653452)]									
		B.50	√	√	——	√		lineages	BA.2.3.7	DY.3	XBB.1.18	XBB.1.9.5	B.50	XBB.1.9	XBB.1.5.49	B.1.1.161	B.1.1.529	XBB.1.44			
		B.50	√	√	XBB.1.5.49	√		abundances	0.25902808	0.17137134	0.09890619	0.0802005	0.05224083	0.04305058	0.03358548	0.02472485	0.01784808	0.01358338			
								resid	12.11606923												
								coverage	99.66210565												
3	M-3	AY.30	BA.5.2.1	BA.2.76	HH.1	EG.2	M-3	2846.46													
		B.1.617.2.30	BA.5.2.1	BA.2.76	XBB.2.3.2.1	XBB.1.9.2.2		summarized	[('Omicron',	0.7571460799661878),	('Delta',	0.1458299330566627),	('Other',	0.02554341272242678)]							
		√	BF=BA.5.2.1.X	——	——	√		lineages	EG.2	AY.30	BF.25	XM	BF.7	HH.1	BA.5	XBB.2.3.11	BA.5.2	B.1.1	XBB.1.5.28	FY.5	XBB.2.3.2
		√	BF25、BF7		√	√		abundances	0.28825317	0.14582993	0.10396513	0.07143909	0.0648234	0.05566293	0.05085629	0.04202304	0.03417595	0.02554341	0.0181622	0.01440661	0.01337829
								resid	17.49972036												
								coverage	99.65541467												
4	M-4	B.1.1.487	BA.5.2.27	JN.1	XBB.1.42.1	XBB.1.9.5	M-4	3293.72													
		B.1.1.487	BA.5.2.27	BA.2.86.1.1	XBB.1.42.1	XBB.1.9.5		summarized	[('Omicron',	0.8167413289141051),	('Other',	0.06087841007414066)]									
		——	√	——	√	√		lineages	XBB.1.42.1	EG.2	BA.5.2.6	BA.5.2.27	XBB.1.9.5	JN.10	XBB	XBV	BA.5	JN.1	XAH	XBB.1.9	
		——	√	√	√	√		abundances	0.17739342	0.13326568	0.09832847	0.08899538	0.0845666	0.06516836	0.06371711	0.06087841	0.04144122	0.03114673	0.02108032	0.01163803	
								resid	11.32067377												
								coverage	99.72901542												
5	M-5	B.1.1.487	BA.5.1.3	BA.2.2.1	GY.1.1	EG.4	M-5	3477.93													
		B.1.1.487	BA.5.1.3	BA.2.2.1	XBB.1.16.2.1.1	XBB.1.9.2.4		summarized	[('Omicron',	0.9278038645392129),	('Other',	0.010972899987863232)]									
		——	√	√	——	√		lineages	BA.2.73	BA.5.1.3	BA.2.2.1	EG.4	BA.2.10	GY.1	GY.1.1	B.1.1.487					
		√	√	√	√	√		abundances	0.24956205	0.21641132	0.210573	0.14938617	0.05027963	0.03904992	0.01254177	0.0109729					
								resid	10.84962687												
								coverage	99.66210565												
6	C1-1	AY.85	B.1.36	AY.30	B.1.1.7	BE.1.1	C1-1	summarized	[('Delta',	0.381433443933112),	('Alpha',	0.19060322240419916),	('Omicron',	0.18770953019018025),	('Other',	0.1399334795867076)]					
		√	√+.27	√	√	√		lineages	AY.85	BE.1.1	B.1.1.7	B.1.36.27	AY.30	Q.7	AY.1	B.1.143					
		√	√+.27	√	√	√		abundances	0.24615889	0.18770953	0.16397482	0.12462686	0.11587868	0.0266284	0.01939587	0.01530662					
								resid	14.91616776												
								coverage	100												
7	C1-2	B.1.1.487	B.1.36	B.1.36	B	BA.5.2.7	C1-2	summarized	[('Other',	0.45986134710291543),	('Omicron',	0.17382078727576097)]									
		√	√+.27	√+.27	√	√		lineages	B.1.36.27	BA.5.2.7	B	B.1.1.487	B.1.1.189	B.1.1.161	BA.5.2.21						
		√	√+.27	√+.27	√	√		abundances	0.34494781	0.16114223	0.05881061	0.023105	0.01798034	0.01501758	0.01267856						
								resid	11.86773911												
								coverage	100												
8	C1-3	B.1.1.487	B	B	B.1.617.2	BA.2.2.1	C1-3	summarized	[('Other',	0.3858259757355056),	('Omicron',	0.3471621720112029),	('Delta',	0.13830072278754357)]							
		√-.487	√	√	√+.29	√		lineages	B.1.1	BA.2.2.1	B	AY.9	XAP	B.1.1.529							
		√-.487	√	√	√+.29	√		abundances	0.24604664	0.207222	0.13977934	0.13830072	0.09312467	0.04681551							
								resid	12.88461646												
								coverage	99.81265264												
9	C1-4	AY.85	B.1.36	AY.85	B.1.1.7	JN.1	C1-4	summarized	[('Delta',	0.46750100000017036),	('Other',	0.1799593483993398),	('Alpha',	0.1799546344897931),	('Omicron',	0.09208399705747791)]					
		√	√+.27	√	√	√		lineages	AY.85	B.1.1.7	B.1.36.27	JN.1	JN.10	B.1.143	Q.7	BA.2					
		√	√+.27	√	√	√		abundances	0.467501	0.16135263	0.1459959	0.04226986	0.03845331	0.03396344	0.018602	0.01136083					
								resid	19.21065208												
								coverage	100												
10	C1-5	B	B	AY.36	B.1.36	BA.2.75.1	C1-5	summarized	[('Other',	0.35682711556871616),	('Omicron',	0.27843107792820765),	('Delta',	0.19511042292369396)]							
		√	√	√	√+.27	√		lineages	BA.2.75.1	B	B.1.36.27	AY.36	AY.1	B.1.1.529							
		√	√	√	√+.27	√		abundances	0.23516187	0.21393912	0.142888	0.141651	0.05345942	0.04326921							
								resid	13.30071832												
								coverage	100												
11	C2-1	B	B.1.36	B.1.1.7	BA.5.2.1	BA.5.1.3	C2-1	summarized	[('Omicron',	0.37810858522821666),	('Other',	0.28801431309839853),	('Alpha',	0.17773476199655625)]							
		√	√+.27	√	√	——		lineages	BA.5.2.1	B.1.36.27	BA.5.3	B.1.1.7	B	BA.5.1.3	Q.7						
		√	√	√	√	√		abundances	0.17699206	0.161333	0.13270907	0.13127296	0.12668131	0.06840745	0.0464618						
								resid	13.41153674												
								coverage	100												
12	C2-2	B	AY.36	B.1.36	BA.1	BE.1.1	C2-2	summarized	[('Omicron',	0.3125098055479885),	('Other',	0.2781763953398677),	('Delta',	0.16853299999440222)]							
		√	√	√+.27	√	√		lineages	BE.1.1	AY.36	B	B.1.36.27	BA.1								
		√	√	√+.27	√	√		abundances	0.20263179	0.168533	0.1408174	0.137359	0.10987801								
								resid	10.50144275												
								coverage	100												
13	C2-3	AY.85	B	B.1.36	BA.1	BA.5.1	C2-3	summarized	[('Other',	0.35199440352370287),	('Delta',	0.3087980000074719),	('Omicron',	0.17010113156836038)]							
		√	√	√+.27	√	——		lineages	AY.85	B	B.1.36.27	BA.1	B.1.143	BA.5.1.6	BA.5.3						
		√	√	√+.27	√	√+.6		abundances	0.308798	0.14344643	0.13974691	0.11476483	0.06880106	0.02939109	0.0259452						
								resid	12.2060864												
								coverage	100												
14	C2-4	B.1.1.7	B	B.1.36	BA.5.1.3	BG.2	C2-4	summarized	[('Other',	0.3572967577215377),	('Omicron',	0.31724797666841953),	('Alpha',	0.21025200502154973)]							
		√	√	√+.27	——	√		lineages	B.1.36.27	B.1.1.7	BA.5.3	B	BG.2	BA.5.1.3	B.1.1.161	Q.7	BA.3				
		√	√	√+.27	√	√		abundances	0.17716	0.16871851	0.14950246	0.12947869	0.07860012	0.06789862	0.05065807	0.0415335	0.02124677				
								resid	12.96847009												
								coverage	100												
15	C2-5	B.1.1.487	B.1.36	AY.36	BA.5.2.7	BE.1.1	C2-5	summarized	[('Omicron',	0.41086522689485083),	('Other',	0.20587927017260454),	('Delta',	0.18332299987724723)]							
		——	√+.27	√	√	√		lineages	BE.1.1	B.1.36.27	AY.36	BA.5.2.7	BA.5.2.8	B.1.533							

		——	√+.27	√	√	√		abundances	0.21538579	0.189087	0.183323	0.17683529	0.01864415	0.01679227						
								resid	11.87066011											
								coverage	100											
16	C3-1	B.1.1.487	BA.2.2.1	BA.2.10	BA.5.2.27	BA.2.75.1	C3-1	summarized	[('Omicron',	0.9231443202478207)]										
		——	√	√	√	√-.1		lineages	BA.5.2.27	BA.2.2.1	BA.2.75	BA.2.10	BA.2.2	BA.2.75.1	XAH	XBD	BA.5			
		——	√	√	√	√		abundances	0.18585591	0.182348	0.14942499	0.10654654	0.09207599	0.06528333	0.05846714	0.05090689	0.03223553			
								resid	6.489635379											
								coverage	99.84276204											
17	C3-2	B.1.36	BA.2.3.7	BA.5.2.7	JN.1.3	BA.2.75.1	C3-2	summarized	[('Omicron',	0.7201466233422218),	('Other',	0.16783339233390127)]								
		√+.27	√	√	√-.3	√		lineages	BA.2.3.7	BA.2.75.1	B.1.36.27	BA.5.2.7	JN.1	BA.2.65	BA.5.2.8	BA.3				
		√+.27	√	√	√-.3	√		abundances	0.23563908	0.1967888	0.16783339	0.12131143	0.07704404	0.03735676	0.03333964	0.01866687				
								resid	12.62601975											
								coverage	100											
18	C3-3	AY.36	BA.5.1	BA.2.3.7	BG.2	BA.2.10	C3-3	summarized	[('Omicron',	0.782625736552486),	('Delta',	0.18837800000616334)]								
		√	√	√	√	√		lineages	BA.2.3.7	AY.36	BA.5.1	BA.2.10	BG.2	XJ	XAH	XE	BA.2.12	BA.5	BA.5.3	
		√	√	√	√	√		abundances	0.266403	0.188378	0.1275029	0.11268825	0.0891302	0.07867812	0.02958619	0.02914365	0.01836622	0.01613895	0.01498825	
								resid	12.1198468											
								coverage	99.90967181											
19	C3-4	B.1.36	BA.2.38	BA.5.2.1	BA.5.2.27	BN.1.2	C3-4	summarized	[('Omicron',	0.7024269082989902),	('Other',	0.19184514064594316)]								
		√+.27	√	√+.28	√	√		lineages	BN.1.2	B.1.36.27	BA.5.2.27	BF.28	BA.2.38	XBD	BA.3					
		√+.27	√	√+.28	√	√		abundances	0.19555445	0.19184514	0.18460378	0.13599178	0.1258915	0.03502579	0.02535961					
								resid	11.37562092											
								coverage	100											
20	C3-5	B	BA.5.2.49	BA.2.2.1	BA.1	BA.2.76	C3-5	summarized	[('Omicron',	0.7948905552687626),	('Other',	0.13823057761200525)]								
		√	√	√	——	——		lineages	BA.2.2.1	XM	BA.5.2.49	B	B.1.1.529	DZ.1	BA.5.2.6	B.1.1.161	BA.1	BA.1.6		
		√	√	√	√	——		abundances	0.2196609	0.2173996	0.12915866	0.10349989	0.07429543	0.06986034	0.05001753	0.03473069	0.01747343	0.01702467		
								resid	11.7074917											
								coverage	99.71563347											
21	C4-1	BA.5.2.7	BA.2.38	BA.5.1.3	DY.3	XBB.1.5.4	C4-1	summarized	[('Omicron',	0.9661868416398551)]										
		√	√	√	√	√		lineages	DY.3	BA.5.2.7	BA.2.38	XBB.1.5.4	BA.5.1.3	XBB.1.4	XAH	BA.5	BA.5.2	BA.5.2.8		
		√	√	√	√	√		abundances	0.22425645	0.187755	0.162162	0.16156747	0.14892508	0.02016377	0.01971354	0.01554885	0.01333695	0.01275773		
								resid	7.136378033											
								coverage	99.24057409											
22	C4-2	BA.5.2.27	DZ.2	BG.2	BE.1.1	HH.1	C4-2	summarized	[('Omicron',	0.9627364752882939)]										
		√	√	√	√	——		lineages	DZ.2	BE.1.1	BA.5.2.27	BG.2	BA.2.65	HH.1	BA.5					
		√	√	√	√	√		abundances	0.25492772	0.24961696	0.227053	0.08692386	0.0690553	0.0455975	0.02956214					
								resid	6.269630773											
								coverage	99.24057409											
23	C4-3	BA.5.1	BE.1.1	BA.5.1.3	BA.1	FL.2	C4-3	summarized	[('Omicron',	0.9591101199224349)]										
		√+.3	√	√	√	√		lineages	FL.2	BE.1.1	BA.5.1.3	XM	BA.1	BA.5.3	BA.5.3.1	BA.1.6				
		√+.3	√	√	√	√		abundances	0.23268755	0.23205519	0.20197686	0.14863133	0.07235535	0.02762413	0.02595022	0.01782949				
								resid	8.258771416											
								coverage	99.71563347											
24	C4-4	BA.5.1	BA.5.2.49	BA.2.75.1	BA.5.1.3	GY.1.1	C4-4	summarized	[('Omicron',	0.9446032735609112)]										
		——	√	√	√	——		lineages	BA.2.75.1	BA.5.1.3	DZ.1	BA.2.10	XAS	BA.5.2.8	BA.5.2.49	GY.1	BA.2.64	BA.2.75	BA.2.12.1	GY.2
		√	√	√	√	√-.1		abundances	0.25421276	0.23498074	0.10373912	0.0846719	0.06039242	0.05048337	0.03608581	0.03105092	0.02851116	0.02590645	0.0179897	0.01657892
								resid	9.938431285											
								coverage	99.24057409											
25	C4-5	BA.5.1.3	BA.2.10	BG.2	BE.1.1	GW.5.3.1	C4-5	summarized	[('Omicron',	0.9794359729441638)]										
		√	√	——	√	√		lineages	BE.1.1	BA.5.1.3	BA.2.10	GW.5.3.1	BA.2.65	GW.5	BG.2	BA.2.12	BA.5.1.12	XAS	XBB.1.19.1	BA.2.12.1
		√	√	√	√	√		abundances	0.21707397	0.15457818	0.1199978	0.115117	0.08631458	0.07521658	0.07184944	0.04969711	0.03385127	0.0279494	0.0155026	0.01228804
								resid	8.700227434											
								coverage	99.83941655											
26	C5-1	BA.5.2.1	BA.5.1.3	BA.5.2.27	XBB.1.16.1	XBB.1.9.5	C5-1	summarized	[('Omicron',	0.9430508794342044)]										
		√+.3	√	√	——	√		lineages	BA.5.1.3	BA.5.2.27	XBB.1.16.11	BF.3	XBB.1.9.5	BA.5.2.4	XBB.1.9	BF.28	XBB.1.16.1	FY.5		
		√+.3	√	√	√	√		abundances	0.1785886	0.1640464	0.15026172	0.08580602	0.0786901	0.07586072	0.0606853	0.05811424	0.047757	0.04324078		
								resid	6.104626099											
								coverage	99.24057409											
27	C5-2	BA.2.3.7	BA.2.10	JN.1	XBB.1.16.1	EG.2	C5-2	summarized	[('Omicron',	0.9626367609872303)]										
		√	√	——	√	√		lineages	BA.2.3.7	XBB.1.16.1	EG.2	BA.2.10	BA.2.1	JN.1	XBB.1.9.2					
		√	√	√	√	√		abundances	0.242808	0.17938343	0.17244788	0.15609039	0.1005769	0.08667896	0.0246512					
								resid	10.69043532											
								coverage	99.89628985											
28	C5-3	BA.5.2.27	BG.2	BA.5.2.49	XBB.1.5.15	XBB.1.42.1	C5-3	summarized	[('Omicron',	0.915426147489491),	('Other',	0.036131552183941644)]								
		√	√	√	√+.2	√		lineages	XBB.1.42.1	BA.5.2.27	FD.2	BA.5.2.49	BG.2	DZ.1	XBV	BA.5	XBB.1.5.15	XBB	BA.5.2.6	
		√	√	√	√+.2	√		abundances	0.22239233	0.22052465	0.18191122	0.08605289	0.07484343	0.06509688	0.03613155	0.02308482	0.01815525	0.0123655	0.01099917	
								resid	6.664668931											
								coverage	99.24057409											
29	C5-4	BG.2	BA.2.2.1	BA.2.10	FE.1.1	XBB.1.9.5	C5-4	summarized	[('Omicron',	0.9572967711480168)]										
		√	√	√	√	——		lineages	BA.2.2.1	FE.1.1	BA.2.10	FL.2.4	BG.2	BA.2.65	XBB.1.18	BA.2.2	XBB.1.9.5			
		√	√	√	√	√		abundances	0.211712	0.16979661	0.12730348	0.09958206	0.0795291	0.07809989	0.06598522	0.063838	0.0614504			
								resid	7.569574172											
								coverage	99.83941655											
30	C5-5	BA.2.38	BA.2.3.7	BE.1.1	FL.13	GW.5.3.1	C5-5	summarized	[('Omicron',	0.916602324320431),	('Other',	0.05474191275908443)]								
		√	√	√	√+.1	——		lineages	BE.1.1	BA.2.3.7	FL.13.1	BA.2.38	BA.2.10	GW.5.3.1	XDE	BA.2.1	FL.13	FL.25	BA.5.3.1	

		√	√	√	√+.1	√		abundances	0.18470767	0.17993168	0.13152	0.12300457	0.1143002	0.07708367	0.05474191	0.04724363	0.02503157	0.02010626	0.01367307		
								resid	8.285413483												
								coverage	99.24057409												
31	C6-1	BA.5.2.7	GA.1	GW.5.3.1	FL.2	XBB.1.42.1	C6-1	summarized	[('Omicron',	0.9124032735406497),	('Other',	0.04843396600319554)]									
		√	√	√	√	√		lineages	FL.2	XBB.1.42.1	GA.1	BA.5.2.7	GW.5.3.1	XDE	XBB.1.19	BA.5.2.6	XBB.1.17.1	XBB.1.19.1	BA.5.2.23		
		√	√	√	√	√		abundances	0.19936228	0.1899798	0.161707	0.13118977	0.0950954	0.04843397	0.03651153	0.0343417	0.02577648	0.02240949	0.01602982		
								resid	6.484723953												
								coverage	99.24057409												
32	C6-2	DZ.2	XBB.1.9.5	XBB.1	GW.5.3.1	XBB.1.42.1	C6-2	summarized	[('Omicron',	0.9028085635548736),	('Other',	0.04638414798297391)]									
		√	√	√+.5.28	√	√		lineages	DZ.2	XBB.1.42.1	XBB.1.5.28	GW.5.3.1	XBB.1.14	XBB.1.5.40	XBB.1.1	XBB.1.9.5	BA.5.2	XDE	XBB.1.5.23	XBB.1.9	EG.2
		√	√	√	√	√		abundances	0.18897432	0.17542771	0.1019822	0.0701092	0.06336807	0.05670855	0.05182661	0.0507937	0.04955658	0.04638415	0.04075924	0.02616111	0.01510392
								resid	9.473359853												
								coverage	99.66210565												
33	C6-3	BA.2.76	GA.1	XBB.1.9.5	XBB.1.42.1	XBB.1.5	C6-3	summarized	[('Omicron',	0.9423161854696444)]											
		√	√	√	√	√+.28		lineages	XBB.1.42.1	GA.1	XBB.1.5.28	XBB.1.9.5	BA.2.76	XBB.1.5.77	XBB.1.17	XBB.1.9	XBB.1.17.1	XBB.1.22			
		√	√	√	√	√+.28		abundances	0.21228635	0.148991	0.0857266	0.0845666	0.08303346	0.07936591	0.0784098	0.07645132	0.06609282	0.02739232			
								resid	5.224898071												
								coverage	99.24057409												
34	C6-4	BA.5.1	XBB.1	FE.1.1	GA.1	FL.13	C6-4	summarized	[('Omicron',	0.9673819027142181)]											
		√	√+.5	√	√	√+.1		lineages	XBB.1.5	GA.1	FE.1.1	BA.5.1	FL.13.1	FL.2.4	BA.5	XBB.1.39	XBB				
		√	√+.5	√	√	√+.1		abundances	0.23349935	0.16269731	0.13966963	0.10578073	0.101047	0.0768053	0.0535326	0.04885701	0.04549298				
								resid	6.802805585												
								coverage	99.66210565												
35	C6-5	BG.2	XBB.1.42.1	HK.3.3	XBB.1	FL.2	C6-5	summarized	[('Omicron',	0.9630353114446671)]											
		√	√	√	√+.5.28	√		lineages	HK.3.3	FL.2	XBB.1.42.1	XBB.1.5.28	BG.2	XBB.1.39	XBB.1	HK.3					
		√	√	√	√	√		abundances	0.212742	0.19975814	0.19256073	0.12826264	0.07323582	0.06399994	0.06180641	0.03066963					
36	T-O-1	B	B.1.1.487	AY.30	B.1.617.2	B.1.36	T-O-1	summarized	[('Other',	0.4364866206968294),	('Delta',	0.2890008239099724)]									
		B	B.1.1.487	B.1.617.2.30	B.1.617.2	B.1.36		lineages	B.1.36.27	AY.30	AY.24	B	B.1.1	B.1.551	B.1						
		√	B.1+B.1.551	√	√+.24	√+.27		abundances	0.15151737	0.14952757	0.13947326	0.08448467	0.08008201	0.06213698	0.05826559						
		√	B.1+B.1.551	√	√+.24	√+.27		resid	8.876707977												
								coverage	100												
37	T-O-2	AY.85	AY.36	B	B.1.617.2	B.1.1.7	T-O-2	summarized	[('Delta',	0.6700167040984318),	('Alpha',	0.17355740700204708),	('Other',	0.10102115430485181)]							
		B.1.617.2.85	B.1.617.2.36	B	B.1.617.2	B.1.1.7		lineages	AY.85	B.1.1.7	B.1.617.2	AY.36	B	AY.9	Q.7	AY.106					
		√	√	√	√	√		abundances	0.29673	0.14200401	0.13995761	0.13856292	0.10102115	0.07866277	0.0315534	0.01610339					
		√	√	√	√	√		resid	10.63130576												
								coverage	99.90298083												
38	T-O-3	B.1.617.2	B	AY.36	AY.30	B.1.36	T-O-3	summarized	[('Delta',	0.4361742775269694),	('Other',	0.3578289703622396)]									
		B.1.617.2	B	B.1.617.2.36	B.1.617.2.30	B.1.36		lineages	B	AY.30	AY.36	B.1.36.27	AY.9	B.1							
		√+.9	√	√	√	√+.27		abundances	0.18455915	0.17613983	0.172596	0.155794	0.08743844	0.01747582							
		√+.9	√	√	√	√+.27		resid	9.606708161												
								coverage	100												
39	T-O-4	B.1.1.487	B.1.1.7	B.1.617.2	B.1.36	AY.30	T-O-4	summarized	[('Other',	0.3014692636398053),	('Delta',	0.2900685811981087),	('Alpha',	0.2365065020085385)]							
		B.1.1.487	B.1.1.7	B.1.617.2	B.1.36	B.1.617.2.30		lineages	B.1.1.7	AY.30	B.1.36.27	AY.9	B.1.551	B.1	Q.7						
		B.1+B.1.551	√	√+.9	√+.27	√		abundances	0.1733384	0.15800221	0.15265614	0.13206637	0.08268694	0.06612618	0.0631681						
		B.1+B.1.551	√	√+.9	√+.27	√		resid	10.99185487												
								coverage	100												
40	T-O-5	B.1.617.2	B.1.1.7	B.1.1.487	AY.30	AY.36	T-O-5	summarized	[('Delta',	0.7011156978150622),	('Alpha',	0.21809509783712383),	('Other',	0.03368179998364558)]							
		B.1.617.2	B.1.1.7	B.1.1.487	B.1.617.2.30	B.1.617.2.36		lineages	AY.36	AY.30	B.1.1.7	B.1.617.2	AY.9	Q.7	B.1.1.487						
		√	√	X	√	√		abundances	0.247664	0.18991547	0.1637018	0.14812005	0.11541617	0.0543933	0.0336818						
		√	√	√	√	√		resid	8.264774461												
								coverage	99.85614399												
41	T-BA-1	BA.5.2.48	JN.1	BA.5.2.1	BA.2.10	JN.1.1	T-BA-1	summarized	[('Omicron',	0.8944726509745479)]											
		BA.5.2.48	BA.2.86.1.1	BA.5.2.1	BA.2.10	BA.2.86.1.1.1		lineages	BA.2.10	BF.25	BA.5.2.48	JN.1.1	BA.2.1	BA.2.56	BA.2.16	JN.3	JN.1	CT.1	BA.5.2.9	JN.8	BA.5.2.8
		√	√-.1	√+.25	√	√		abundances	0.1373305	0.12714003	0.10690852	0.10521356	0.077996	0.05937536	0.05654601	0.04756187	0.0461708	0.03799002	0.03170054	0.0306653	0.02987413
		√	√	√+.25	√	√		resid	14.16768055												
								coverage	99.89628985												
42	T-BA-2	BG.2	DY.3	JN.1.1	BA.5.2.27	JN.1.3	T-BA-2	summarized	[('Omicron',	0.9787435501809867)]											
		BA.2.12.1.2	BA.5.2.48.3	BA.2.86.1.1.1	BA.5.2.27	BA.2.86.1.1.3		lineages	DY.3	XAS	JN.1.1	BA.5.2.27	BG.2	JN.3	BA.2.65	JN.10	BA.2.86.1				
		√	√	√	√	JN.1.1		abundances	0.25444789	0.18961847	0.17281396	0.16552575	0.09381627	0.05058857	0.02546783	0.014729	0.01173582				
		√	√	√	√	JN.1.1		resid	14.18306557												
								coverage	99.72566993												
43	T-BA-3	BA.5.1.3	BG.2	BE.1.1	DY.1	DZ.2	T-BA-3	summarized	[('Omicron',	0.9762001270966906)]											
		BA.5.1.3	BA.2.12.1.2	BA.5.3.1.1.1	BA.5.2.48.1	BA.5.2.49.2		lineages	DZ.2	BE.1.1	DY.1	BA.5.1.3	BG.2	BA.5.2	XAS	BA.2.65	BA.5.3	BA.5	BA.2.12	BA.5.2.59	
		√	√	√	√	√		abundances	0.22466177	0.19784601	0.18977295	0.12130919	0.06937039	0.05857305	0.0408857	0.01778967	0.01663876	0.01654271	0.01196494	0.01084501	
		√	√	√	√	√		resid	5.223142034												
								coverage	99.24057409												
44	T-BA-4	DY.3	JN.1	BA.5.2.1	BF.7.14	BA.2.3.7	T-BA-4	summarized	[('Omicron',	0.9294546493597126)]											
		BA.5.2.48.3	BA.2.86.1.1	BA.5.2.1	BA.5.2.1.7.14	BA.2.3.7		lineages	BA.2.3.7	DY.3	BA.2.3	BF.7.14.6	JN.1	BF.7.14	BF.28	BA.2.1	BA.2.86.1				
		√	√	BF.7.14.6	√+.6	√		abundances	0.248793	0.236102	0.13553186	0.1195046	0.07349447	0.04528763	0.03328135	0.0250589	0.01240084				
		√	√	BF.7.14.6+BF.7.14	√+.6	√		resid	11.01379641												
								coverage	99.72901542												
45	T-BA-5	BE.1.1	BA.2.2.1	JN.1.3	BG.2	BN.1.2	T-BA-5	summarized	[('Omicron',	0.9658651110713212)]											
		BA.5.3.1.1.1	BA.2.2.1	BA.2.86.1.1.3	BA.2.12.1.2	BA.2.75.5.1.2		lineages	BN.1.2	BA.2.2.1	BE.1.1	BA.2.65	JN.1	BG.2	JN.3						
		√	√	JN.1	——	√		abundances	0.24984843	0.226729	0.21687522	0.09981632	0.08018381	0.07455413	0.0178582						

		√	√	JN.1	√	√			resid	9.115234643								
									coverage	99.70225151								
46	T-XBB-1	GA.1	FL.15.2	FU.1	GF.1	EG.4		T-XBB-1	summarized	[('Omicron',	0.9919447888727193)]							
		XBB.1.17.1.1	XBB.1.9.1.15.2	XBB.1.16.1.1	XBB.1.5.24.1	XBB.1.9.2.4			lineages	FL.15.2	FU.1	GF.1	GA.1	XBB.1.22	EG.4	EG.4.5		
		√	√	√	√	——			abundances	0.207345	0.186711	0.17371	0.171276	0.10654398	0.10591516	0.04044364		
		√	√	√	√	√			resid	5.34945549								
									coverage	99.66210565								
47	T-XBB-2	XBB.1.5.15	EG.2	XBB.1.42.1	XBB.1.16.7	XBB.1.5.7		T-XBB-2	summarized	[('Omicron',	0.9560780928356013),	('Other',	0.028678296712109985)]					
		XBB.1.5.15	XBB.1.9.2.2	XBB.1.42.1	XBB.1.16.7	XBB.1.5.7			lineages	EG.2	XBB.1.16.7	XBB.1.42.1	FD.2	XBB.1.5.7	XBB.1.5.28	XBB.1.5.15	XDB	FL.26
		——	√	√	√	√			abundances	0.1892664	0.187998	0.18741823	0.13909135	0.10460048	0.07861877	0.04313545	0.0286783	0.02594943
		√	√	√	√	√			resid	5.390827566								
									coverage	99.24057409								
48	T-XBB-3	XBB.1.5.7	XBB.1.9.5	HK.3.3	XBB.1.16.3	XBB.1.16.7		T-XBB-3	summarized	[('Omicron',	0.9723444726473409)]							
		XBB.1.5.7	XBB.1.9.5	XBB.1.9.2.5.1.1.3.3	XBB.1.16.3	XBB.1.16.7			lineages	HK.3.3	XBB.1.5.7	XBB.1.16.7	XBB.1.16.11	XBB.1.9.5	XBB.1.16.3	XBB.1.9	XBB.1.38	
		√	√	√	——	√			abundances	0.22395934	0.20642279	0.18443873	0.09168624	0.0893356	0.0740173	0.07026219	0.03222227	
		√	√	√	√	√			resid	5.378501768								
									coverage	99.24057409								
49	T-XBB-4	FL.2	EG.4	XBB.1.5.15	XBB.1.16.7	GY.1.1		T-XBB-4	summarized	[('Omicron',	0.7737603539733726),	('Other',	0.20627116099277526)]					
		XBB.1.9.1.2	XBB.1.9.2.4	XBB.1.5.15	XBB.1.16.7	XBB.1.16.2.1.1			lineages	FL.2	XDB	EG.4	XBB.1.16.7	FD.2	XBB.1.5.15	GY.1	GY.1.1	
		√	√	——	√	——			abundances	0.21595558	0.20627116	0.1792704	0.159914	0.10826674	0.07093653	0.02771765	0.01169945	
		√	√	√	√	√			resid	5.725339508								
									coverage	99.66210565								
50	T-XBB-5	XBB.1.16.7	XBB.1.5.15	FU.1	HH.1	GF.1		T-XBB-5	summarized	[('Omicron',	0.9720909248568897)]							
		XBB.1.16.7	XBB.1.5.15	XBB.1.16.1.1	XBB.2.3.2.1	XBB.1.5.24.1			lineages	FU.1	XBB.1.16.7	GF.1	FD.2	XBB.1.5.57	XBB.1.38	HH.1	XBB.2.3.11	XBB.1.5.15
		√	——	√	——	√			abundances	0.22659924	0.2030749	0.180876	0.15541526	0.08307925	0.03618252	0.03295695	0.0299574	0.02394941
		√	√	√	√	√			resid	5.907637161								
									coverage	99.24057409								
51	S-Delta	B.1.617.2	AY.30	AY.36	AY.85			S-Delta										
		B.1.617.2	B.1.617.2.30	B.1.617.2.36	B.1.617.2.85				summarized	[('Delta',	0.960891295556471)]							
		√+.9	√	√	√				lineages	AY.85	AY.36	AY.30	AY.9	B.1.617.2	AY.106			
		√	√	√	√				abundances	0.350789	0.20138653	0.17924521	0.12958701	0.059123	0.04076055			
									resid	8.96947828								
									coverage	99.85614399								
52	S-XBB.1.5	XBB.1.5	XBB.1.5.4	XBB.1.5.7	XBB.1.5.15	GF.1	XBB.1.5.59	S-XBB.1.5										
		XBB.1.5	XBB.1.5.4	XBB.1.5.7	XBB.1.5.15	XBB.1.5.24.1	XBB.1.5.59		summarized	[('Omicron',	0.9805385210723057)]							
		√+.52	——	√	√+.2	√	√		lineages	XBB.1.5.52	XBB.1.5.7	GF.1	FD.2	XBB.1.5.59	XBB.1.5.4	XBB.1.5.57	XBB.1.5.15	
		√+.52	√	√	√+.2	√	√		abundances	0.251357	0.23939399	0.136602	0.12675504	0.07619023	0.07490757	0.05746814	0.01786456	
									resid	5.241472354								
									coverage	99.24057409								
53	S-XBB.1.9.1	FL.2	FL.5	FL.13	FL.15.2	FL.37		S-XBB.1.9.1										
		XBB.1.9.1.2	XBB.1.9.1.5	XBB.1.9.1.13	XBB.1.9.1.15.2	XBB.1.9.1.37			summarized	[('Omicron',	0.9963364636459155)]							
		√	√	√+.1	√	√			lineages	FL.2	FL.15.2	FL.13.1	FL.5	FL.37				
		√	√	√+.1	√	√			abundances	0.43289885	0.232957	0.182171	0.11084192	0.0374677				
									resid	4.374682919								
									coverage	99.24057409								
54	S-XBB.1.9.2	EG.2	EG.4	JG.3	HK.3.3			S-XBB.1.9.2										
		XBB.1.9.2.2	XBB.1.9.2.4	XBB.1.9.2.5.1.3.3	XBB.1.9.2.5.1.1.3.3				summarized	[('Omicron',	0.9925173036293141)]							
		√	√+.5	√	√				lineages	HK.3.3	EG.4.5	EG.2	JG.3	HK.3	EG.4	XBB.1.9.2	EG.5.1.3	
		√	√+.5	√	√				abundances	0.306778	0.23217819	0.16433176	0.14210117	0.05749172	0.04036108	0.03767566	0.01159971	
									resid	3.730787777								
									coverage	99.66210565								
55	S-XBB.1.16	XBB.1.16.1	FU.1	GY.1.1	XBB.1.16.3	XBB.1.16.7		S-XBB.1.16										
		XBB.1.16.1	XBB.1.16.1.1	XBB.1.16.2.1.1	XBB.1.16.3	XBB.1.16.7			summarized	[('Omicron',	0.9804732179359572)]							
		√	√	√-.1.1	√	√			lineages	XBB.1.16.1	FU.1	XBB.1.16.7	XBB.1.16.3	XBB.1.16.2	XBB.1.16.8	GY.1		
		√	√	√-.1	√	√			abundances	0.34338105	0.23132692	0.180227	0.0997926	0.05494137	0.047046	0.02375828		
									resid	4.183898482								
									coverage	99.24057409								
56	S-JN	JN.1	JN.1.1	JN.1.3				S-JN										
		BA.2.86.1.1	BA.2.86.1.1.1	BA.2.86.1.1.3					summarized	[('Omicron',	0.9928778495553126)]							
		√	√	——					lineages	JN.1	JN.1.1							
		√	√	——					abundances	0.66123224	0.33164561							
									resid	6.662507217								
									coverage	99.85614399								
57	S-BA.5.2	BA.5.2.1	BF.7.14	BA.5.2.7	BA.5.2.27	BA.5.2.48	BA.5.2.49	S-BA.5.2										
		BA.5.2.1	BA.5.2.1.7.14	BA.5.2.7	BA.5.2.27	BA.5.2.48	BA.5.2.49		summarized	[('Omicron',	0.9451819686534857)]							
		√+.28	√+.6	√	√	√+.3	√+.1		lineages	BA.5.2.27	BF.28	BA.5.2.7	DY.3	BF.7.14.6	DZ.1	BF.7.14		
		√+.28	√+.6	√	√	√+.3	√+.1		abundances	0.21092499	0.16725912	0.154764	0.12968135	0.10453124	0.0927509	0.08527037		
									resid	6.686804195								
									coverage	99.66210565								
58	S-BA.5.2.48+49	BA.5.2.48	DY.1	DY.2	DY.3	BA.5.2.49	DZ.2	S-BA.5.2.48+49										
		BA.5.2.48	BA.5.2.48.1	BA.5.2.48.2	BA.5.2.48.3	BA.5.2.49	BA.5.2.49.2		summarized	[('Omicron',	0.9718594588904457)]							
		√+?	√	√	√	√+.1	√		lineages	DY.2	DY.3	DZ.2	DY.1	DZ.1	BA.5.2.44			
		√+?	√	√	√	√+.1	√		abundances	0.278361	0.254268	0.175951	0.15907224	0.078211	0.02599622			
									resid	6.082378367								
									coverage	99.66210565								
59	2023-19-CX251	XBF						2023-19-CX251	summarized	[('Omicron', 0.9938316719814522)]								
		√							lineages	XBF								
		√							abundances	0.99383167								
									resid	8.997579584								
									coverage	99.23388311								
60	2023-19-CX611	XBL.3						2023-19-CX611	summarized	[('Other',	0.9987283679677964)]							
		√							lineages	XBL.3	XBL							
		√							abundances	0.89799046	0.10073791							

					resid	4.64860405				
					coverage	99.39112107				
61	2023-19-CX2945	XBC.1.6.2		2023-19-CX2945	summarized	[('Other',	0.9991030409988897)]			
		√			lineages	XBC.1.6.1				
		√			abundances	0.99910304				
					resid	4.847309135				
					coverage	96.07239637				
62	XBG-1	BA.2.76	BA.5.2.7	XBG-1						
		√	√		summarized	[('Omicron',	0.9828064695337266)]			
		√	√		lineages	BA.5.2.7	BA.2.76	BA.5.2.6	BF.7	
					abundances	0.67153261	0.22361212	0.05309968	0.03456206	
					resid	5.261731841				
					coverage	99.24057409				
63	XBG-2	BA.2.76	DY.3	XBG-2						
		√	√		summarized	[('Omicron',	0.9816104146771814)]			
		√	√		lineages	DY.3	BA.2.76	BA.5.2.8	BA.5.2.6	BA.2.21
					abundances	0.696521	0.18806666	0.05747069	0.02074663	0.01880544
					resid	7.214499616				
					coverage	99.2372286				
64	XBG-3	BA.2.76	DZ.2	XBG-3						
		√	√		summarized	[('Omicron',	0.9755471473942124)]			
		√	√		lineages	DZ.2	BA.2.76	BF.7.8	BA.5.2.49	BA.5.2
					abundances	0.710714	0.17746544	0.04801697	0.028768	0.01058274
					resid	6.811211485				
					coverage	98.94951658				
65	XBC-1	BA.2.3.7	B.1.617.2	XBC-1						
		√	√		summarized	[('Omicron',	0.674413431160793),	('Delta',	0.28621493795523584)]	
		√	√		lineages	BA.2.3.7	AY.71	XAC	B.1.1.529	XAH
					abundances	0.48059	0.28621494	0.085504	0.06291834	0.04540109
					resid	17.89729333				
					coverage	99.78923422				
66	XBC-2	BA.2.3.7	AY.36	XBC-2						
		√	√		summarized	[('Omicron',	0.6046781675687839),	('Delta',	0.3828893309450955)]	
		√	√		lineages	BA.2.3.7	AY.36	XAH	XAT	XE
					abundances	0.412766	0.38288933	0.12632665	0.03975602	0.02582949
					resid	16.91366773				
					coverage	99.8829079				
67	XBC-3	BA.2.3.7	AY.30	XBC-3						
		√	√		summarized	[('Omicron',	0.6537571078871127),	('Delta',	0.2798833450220262)]	
		√	√		lineages	BA.2.3.7	AY.30	XM		
					abundances	0.56310596	0.27988335	0.09065115		
					resid	20.62633746				
					coverage	99.65541467				
68	XDD-1	EG.5.1.1	JN.1	XDD-1						
		√	√		summarized	[('Omicron',	0.9950417626354994)]			
		√	√		lineages	EG.5.1.1	JN.1	BA.2.86.1		
					abundances	0.63530975	0.242105	0.11762701		
					resid	17.62939286				
					coverage	99.72566993				
69	XDD-2	EG.5.1.1	JN.1.1	XDD-2						
		√	——		summarized	[('Omicron',	0.9925040728068011)]			
		√	√		lineages	EG.5.1.1	BA.2.86.1	JN.1.1		
					abundances	0.59656207	0.219731	0.176211		
					resid	17.53110218				
					coverage	99.72566993				
70	XDD-3	EG.5.1.1	JN.1.3	XDD-3						
		√	——		summarized	[('Omicron',	0.9924217459963304)]			
		√	——		lineages	EG.5.1.1	JN.1	BA.2.86.1	JN.10	JN.3
					abundances	0.67941148	0.15006142	0.075057	0.049576	0.03831585
					resid	16.78885854				
					coverage	99.70225151				

Supplementary Table 3. The original genotypes and the recovered genotypes of 3 real samples in routine tests.

Named sample	Detection date	Original sample	Genotypes (single sequencing)			Genotypes (pooled sequencing)				
mix-S12	2023/5/24-25	RY1126	EG.5.1.1	XBB.1.9.2.5.1.1	lineages	EG.5.1.1	FL.2.4	EG.5		
		RY1177	EG.5.1.1	XBB.1.9.2.5.1.1	abundances	63.97%	33.70%	1.41%		
		RY1218	FL.2.4	XBB.1.9.1.2.4	resid	2.13444				
					coverage	99.14%				
test-mix1	2023/9/5	2702	FL.15	XBB.1.9.1.15	lineages	FY.3.3	EG.5.1.1	FL.15.2	XBB.1.16.12	XBB.1.16
		2706	XBB.1.16	XBB.1.16	abundances	34.56%	19.60%	16.60%	9.46%	7.07%
		2713	EG.5.1.1	XBB.1.9.2.5.1.1	resid	4.44507				
		2742	FY.3	XBB.1.22.1.3	coverage	99.24%				
test-mix2	2023/9/5	2874	FL.4.5	XBB.1.9.1.4.5	lineages	EG.5.1.1	FL.4.5	JJ.1	EG.5.1.4	
		2896	EG.5.1.4	XBB.1.9.2.5.1.4	abundances	45.84%	28.28%	18.11%	5.49%	
		2928	EG.5.1.1	XBB.1.9.2.5.1.1	resid	4.03265				
		2947	HK.3	XBB.1.9.2.5.1.1.3	coverage	99.24%				