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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	(χ^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	

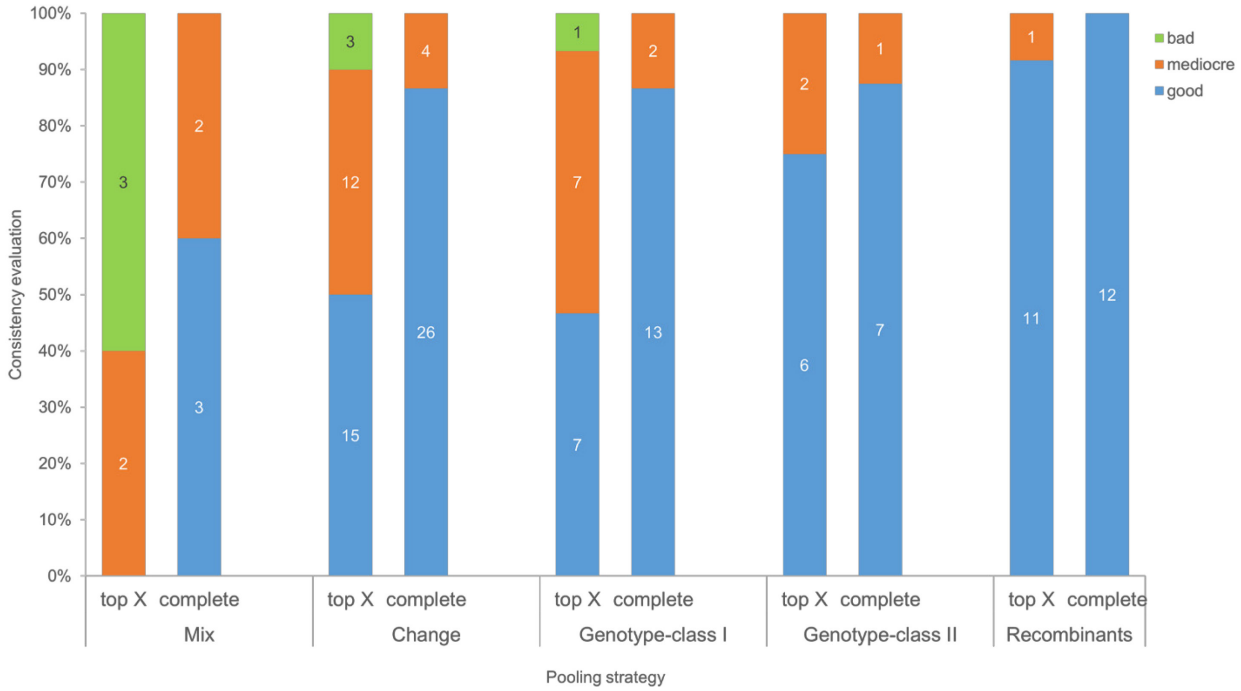
#, Pearson's Chi-squared test; *, Fisher's exact test.

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).

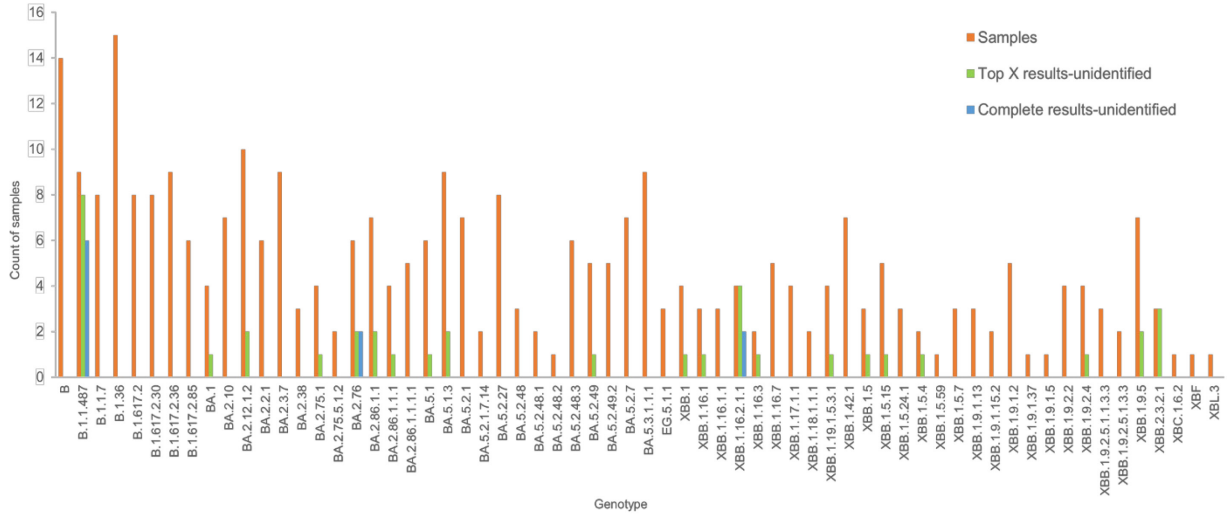
Identification efficiency among different genotypes

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',	0.747991806212 6736),	('Alpha',	0.1921229949875 294)]									
		√	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	0.107868 12	0.10358644	0.09602 485	0.07438334	0.06180 715	0.05509775	0.05337 512	0.03120 199	0.02315 13	0.02271 817	0.01808 24
								resid coverage	15.07037287 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',	0.717573625424 9501),	('Other',	0.0769656771765 3452)]									
		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	0.098906 19	0.0802005	0.05224 083	0.04305058	0.03358 548	0.02472485	0.01784 808	0.01358 338			
								resid coverage	12.11606923 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',	0.757146079966 1878),	('Delta',	0.1458299330566 627),	('Other',	0.025543412722 42678)]							
		√	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	0.103965 13	0.07143909	0.06482 34	0.05566293	0.05085 629	0.04202304	0.03417 595	0.02554 341	0.01816 22	0.01440 661	0.01337 829
								resid coverage	17.49972036 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',	0.816741328914 1051),	('Other',	0.0608784100741 4066)]									
		——	√	——	√	√		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	0.098328 47	0.08899538	0.08456 66	0.06516836	0.06371 711	0.06087841	0.04144 122	0.03114 673	0.02108 032	0.01163 803	
								resid coverage	11.32067377 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',	0.927803864539 2129),	('Other',	0.0109728999878 63232)]									
		——	√	√	——	√		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	0.05027 963	0.03904992	0.01254 177	0.0109729					
								resid coverage	10.84962687 99.66210565												
6	C1-1	AY.85	B.1.36	AY.30	B.1.1.7	BE.1.1	C1-1	summarized	[('Delta',	0.381433443933 112),	('Alpha',	0.1906032224041 9916),	('Omicron',	0.187709530190 18025),	('Other',	0.139933479586 7076)]					
		√	√+.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	0.163974 82	0.12462686	0.11587 868	0.0266284	0.01939 587	0.01530662					
								resid coverage	14.91616776 100												
7	C1-2	B.1.1.487	B.1.36	B.1.36	B	BA.5.2.7	C1-2	summarized	[('Other',	0.459861347102 91543),	('Omicron',	0.1738207872757 6097)]									
		√	√+.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	0.058810 61	0.023105	0.01798 034	0.01501758	0.01267 856						
								resid coverage	11.86773911 100												
8	C1-3	B.1.1.487	B	B	B.1.617.2	BA.2.2.1	C1-3	summarized	[('Other',	0.385825975735 5056),	('Omicron',	0.3471621720112 029),	('Delta',	0.138300722787 54357)]							
		√-.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	0.139779 34	0.13830072	0.09312 467	0.04681551							
								resid coverage	12.88461646 99.81265264												
9	C1-4	AY.85	B.1.36	AY.85	B.1.1.7	JN.1	C1-4	summarized	[('Delta',	0.467501000000 17036),	('Other',	0.1799593483993 398),	('Alpha',	0.179954634489 7931),	('Omicron',	0.092083997057 47791)]					
		√	√+.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	0.145995 9	0.04226986	0.03845 331	0.03396344	0.01860 2	0.01136083					
								resid coverage	19.21065208 100												
10	C1-5	B	B	AY.36	B.1.36	BA.2.75.1	C1-5	summarized	[('Other',	0.356827115568 71616),	('Omicron',	0.2784310779282 0765),	('Delta',	0.195110422923 69396)]							
		√	√	√	√+.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	0.05345 942	0.04326921							
								resid coverage	13.30071832 100												
11	C2-1	B	B.1.36	B.1.1.7	BA.5.2.1	BA.5.1.3	C2-1	summarized	[('Omicron',	0.378108585228 21666),	('Other',	0.2880143130983 9853),	('Alpha',	0.177734761996 55625)]							
		√	√+.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	0.132709 07	0.13127296	0.12668 131	0.06840745	0.04646 18						
								resid coverage	13.41153674 100												
12	C2-2	B	AY.36	B.1.36	BA.1	BE.1.1	C2-2	summarized	[('Omicron',	0.312509805547 9885),	('Other',	0.2781763953398 677),	('Delta',	0.168532999994 40222)]							
		√	√	√+.27	√	√		lineages	BE.1.1	AY.36	B	B.1.36.27	BA.1								
		√	√	√+.27	√	√		abundances	0.20263179	0.168533	0.140817 4	0.137359	0.10987 801								
								resid coverage	10.50144275 100												
13	C2-3	AY.85	B	B.1.36	BA.1	BA.5.1	C2-3	summarized	[('Other',	0.351994403523 70287),	('Delta',	0.3087980000074 719),	('Omicron',	0.170101131568 36038)]							
		√	√	√+.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	0.139746 91	0.11476483	0.06880 106	0.02939109	0.02594 52						
								resid coverage	12.2060864 100												
14	C2-4	B.1.1.7	B	B.1.36	BA.5.1.3	BG.2	C2-4	summarized	[('Other',	0.357296757721 5377),	('Omicron',	0.3172479766684 1953),	('Alpha',	0.210252005021 54973)]							
		√	√	√+.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	0.149502 46	0.12947869	0.07860 012	0.06789862	0.05065 807	0.0415335	0.02124 677				
								resid coverage	12.96847009 100												
15	C2-5	B.1.1.487	B.1.36	AY.36	BA.5.2.7	BE.1.1	C2-5	summarized	[('Omicron',	0.410865226894 85083),	('Other',	0.2058792701726 0454),	('Delta',	0.183322999877 24723)]							
		——	√+.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',	0.7826257365524 86),	('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	0.01879797	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	0.11999978	0.115117	0.08631458	0.07521658	0.07184944	0.04969711	0.03385127	0.02794 94	0.01550 26	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	0.07869 01	0.07586072	0.06068 53	0.05811424	0.04775 7	0.04324 078			
								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	0.10057 69	0.08667896	0.02465 12						
								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',	0.9154261474894491),	('Other',	0.03613155218396 41644)]									
		√	√	√	√+.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	0.01236 55	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	0.07952 91	0.07809989	0.06598522	0.063838	0.06145 04				
								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',	0.9166023243204 31),	('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	0.11430 02	0.07708367	0.05474 191	0.04724363	0.02503 157	0.02010 626	0.01367 307		
								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	0.09509 54	0.04843397	0.03651 153	0.0343417	0.02577 648	0.02240 949	0.01602 982		
								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	0.06336 807	0.05670855	0.05182 661	0.0507937	0.04955 658	0.04638 415	0.04075 924	0.02616 111	0.01510 392
								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	0.08303 346	0.07936591	0.07840 98	0.07645132	0.06609 282	0.02739 232			
								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	0.1396696 3	0.10578073	0.10104 7	0.0768053	0.05353 26	0.04885701	0.04549 298				
								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	0.1925607 3	0.12826264	0.07323 582	0.06399994	0.06180 641	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',	0.28900082390997 24)]									
		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	0.1394732 6	0.08448467	0.08008 201	0.06213698	0.05826 559						
		√	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	0.1399576 1	0.13856292	0.10102 115	0.07866277	0.03155 34	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',	0.35782897036223 96)]									
		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	0.08743 844	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',	0.29006858119810 87),	('Alpha',	0.23650650200855385)]							
		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	0.1526561 4	0.13206637	0.08268 694	0.06612618	0.06316 81						
		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	0.11541 617	0.0543933	0.03368 18						
		√	√	√	√	√		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	0.1069085 2	0.10521356	0.07799 6	0.05937536	0.05654 601	0.04756187	0.04617 08	0.03799 002	0.03170 054	0.03066 53	0.02987 413
		√	√	√+.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	0.1728139 6	0.16552575	0.09381 627	0.05058857	0.02546 783	0.014729	0.01173 582				
		√	√	√	√	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	0.1897729 5	0.12130919	0.06937 039	0.05857305	0.04088 57	0.01778967	0.01663 876	0.01654 271	0.01196 494	0.01084 501	
		√	√	√	√	√		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	0.1355318 6	0.1195046	0.07349 447	0.04528763	0.03328 135	0.0250589	0.01240 084				
		√	√	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	0.2168752 2	0.09981632	0.08018 381	0.07455413	0.01785 82						

		√	√	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',	0.991944788872 7193)]							
		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',	0.956078092835 6013),	('Other',	0.0286782967121 09985)]					
		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',	0.972344472647 3409)]							
		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	0.08933056	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',	0.773760353973 3726),	('Other',	0.2062711609927 7526)]					
		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.1.5.1.5	FU.1	HH.1	GF.1		T-XBB-5	summarized	[('Omicron',	0.972090924856 8897)]							
		XBB.1.16.7	XBB.1.5.1.5	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',	0.960891295556 471)]							
		√+.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',	0.980538521072 3057)]							
		√+.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.1.9.1.17			summarized	[('Omicron',	0.996336463645 9155)]							
		√	√	√+.1	√	√			lineages	FL.2	FL.15.2	FL.13.1	FL.5	FL.37				
		√	√	√+.1	√	√			abundances	0.43289885	0.232957	0.182171	0.11084192	0.03746 77				
									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',	0.992517303629 3141)]							
		√	√+.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',	0.980473217935 9572)]							
		√	√	√-.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',	0.992877849555 3126)]							
		√	√	——					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',	0.945181968653 4857)]							
		√+.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',	0.971859458890 4457)]							
		√+?	√	√	√	√+.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',	0.993831671981452 2)]							
		√							lineages	XBF								
		√							abundances	0.99383167								
									resid	8.997579584								
									coverage	99.23388311								
60	2023-19-CX611	XBL.3						2023-19-CX611	summarized	[('Other',	0.998728367967 7964)]							
		√							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',	0.999103040998 8897)]			
		√			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',	0.982806469533 7266)]			
		√	√		lineages	BA.5.2.7	BA.2.76	BA.5.2.6	BF.7	
					abundances	0.67153261	0.22361212	0.053099 68	0.03456206	
					resid	5.261731841				
					coverage	99.24057409				
63	XBG-2	BA.2.76	DY.3	XBG-2						
		√	√		summarized	[('Omicron',	0.981610414677 1814)]			
		√	√		lineages	DY.3	BA.2.76	BA.5.2.8	BA.5.2.6	BA.2.21
					abundances	0.696521	0.18806666	0.057470 69	0.02074663	0.01880 544
					resid	7.214499616				
					coverage	99.2372286				
64	XBG-3	BA.2.76	DZ.2	XBG-3						
		√	√		summarized	[('Omicron',	0.975547147394 2124)]			
		√	√		lineages	DZ.2	BA.2.76	BF.7.8	BA.5.2.49	BA.5.2
					abundances	0.710714	0.17746544	0.048016 97	0.028768	0.01058 274
					resid	6.811211485				
					coverage	98.94951658				
65	XBC-1	BA.2.3.7	B.1.617.2	XBC-1						
		√	√		summarized	[('Omicron',	0.674413431160 793),	('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	0.04540 109
					resid	17.89729333				
					coverage	99.78923422				
66	XBC-2	BA.2.3.7	AY.36	XBC-2						
		√	√		summarized	[('Omicron',	0.604678167568 7839),	('Delta',	0.38288933094509 55)]	
		√	√		lineages	BA.2.3.7	AY.36	XAH	XAT	XE
					abundances	0.412766	0.38288933	0.126326 65	0.03975602	0.02582 949
					resid	16.91366773				
					coverage	99.8829079				
67	XBC-3	BA.2.3.7	AY.30	XBC-3						
		√	√		summarized	[('Omicron',	0.653757107887 1127),	('Delta',	0.27988334502202 62)]	
		√	√		lineages	BA.2.3.7	AY.30	XM		
					abundances	0.56310596	0.27988335	0.090651 15		
					resid	20.62633746				
					coverage	99.65541467				
68	XDD-1	EG.5.1.1	JN.1	XDD-1						
		√	√		summarized	[('Omicron',	0.995041762635 4994)]			
		√	√		lineages	EG.5.1.1	JN.1	BA.2.86.1		
					abundances	0.63530975	0.242105	0.117627 01		
					resid	17.62939286				
					coverage	99.72566993				
69	XDD-2	EG.5.1.1	JN.1.1	XDD-2						
		√	——		summarized	[('Omicron',	0.992504072806 8011)]			
		√	√		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',	0.992421745996 3304)]			
		√	——		lineages	EG.5.1.1	JN.1	BA.2.86.1	JN.10	JN.3
					abundances	0.67941148	0.15006142	0.075057	0.049576	0.03831 585
					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%				