JCM Accepted Manuscript Posted Online 10 November 2021
J Clin Microbiol doi:10.1128/JCM.01698-21

External quality assessment of SARS-CoV-2-sequencing: An ESGMD-SSM pilot trial across 15 European laboratories

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Abstract

Objective: This first pilot on external quality assessment (EQA) of SARS-CoV-2 whole genome sequencing, initiated by the ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD) and Swiss Society for Microbiology (SSM), aims to build a framework between laboratories in order to improve pathogen surveillance sequencing.

Methods: Ten samples with varying viral loads were sent out to 15 clinical laboratories who had free choice of sequencing methods and bioinformatic analyses. The key aspects on which the individual centres were compared on were identification of 1) SNPs and indels, 2) Pango lineages, and 3) clusters between samples.

Results: The participating laboratories used a wide array of methods and analysis pipelines. Most were able to generate whole genomes for all samples. Genomes were sequenced to varying depth (up to 100-fold difference across centres). There was a very good consensus regarding the majority of reporting criteria, but there were a few discrepancies in lineage and cluster assignment. Additionally, there were inconsistencies in variant calling. The main reasons for discrepancies were missing data, bioinformatic choices, and interpretation of data.

Conclusions: The pilot EQA was an overall success. It was able to show the high quality of participating labs and provide valuable feedback in cases where problems occurred, thereby improving the sequencing setup of laboratories. A larger follow-up EQA should, however, improve on defining the variables and format of the report. Additionally, contamination and/or minority variants should be a further aspect of assessment.

Introduction

Whole genome sequencing (WGS) of SARS-CoV-2 isolates has been used in many countries mainly to determine (i) specific viral lineages and (ii) the molecular epidemiological context. WGS will become increasingly important both as a typing technology also in virological routine diagnostics of individual patients, and for epidemiological surveillance. The European Centre for Disease Prevention and Control (ECDC) has recently published a document to support the usage and implementation of WGS of SARS-CoV-2 in European countries (1).

Quality management is a central element for ensuring accurate and robust laboratory results for both routine diagnostic and reference laboratories. Internal and external controls are integral to the assessment of quality, e.g. in an ISO accredited environment. In particular, external quality assessments (EQAs) represent a corner stone in introducing new test methods, capacity building, and ensuring a baseline quality level. This is even more important in a pandemic situation, when a novel, previously unknown pathogen necessitates prompt development, validation and roll out of assays for which microbiological expertise and diagnostic knowledge are limited. In this context, EQAs can ensure and improve testing quality and results comparability. They also allow, if sufficiently scaled, the comparison of test performance of in-house developed and commercial assays.

To date, no EQA results have been published focusing on WGS of SARS-CoV-2, although some publications have shared quality aspects of single centre's experiences (2,3). Along, these lines, individual centres in Switzerland have published protocols on WGS with

different epidemiological questions (4,5). In the past, the Swiss Institute of Bioinformatics has coordinated an EQA for viral metagenomics (6) and bacterial typing (7) which is an important first step in capacity forming of WGS technology between diagnostic laboratories. Many other European countries are following suit.

For this reason, the ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD) and the Swiss Society of Microbiology (SSM) aimed to conduct a first EQA pilot focusing on SARS-CoV-2 WGS with focus on three key aspects of genome analysis:

(i) identification of SNPs and deletions,

(ii) identification of Pango lineages (8), and

(iii) assessing the genomic relatedness using a molecular epidemiological approach.

The aim is to exchange knowledge and build a framework between the diagnostic laboratories in order to improve the quality for the continuing demands for high quality genomes to address epidemiological questions during an ongoing pandemic.

Methods and Materials

Design of the external quality assessment

The EQA was designed such that each lab could choose its own sequencing method as well as bioinformatic analysis. This introduces variability and makes disentangling methodological effects harder, but reflects best clinical reality. Moreover, it provides direct feedback to laboratories concerning their sequencing pipeline.

An overview of the individual analysis pipelines is shown in **table 1** and a full description can be found in the supplementary materials.

The desired key aspects for the EQA (SNPs/indels, Pango lineage assignment, and cluster assignment) as well as additional features such as read depth and percentage of missing data were reported back to the sequencing team at the University Hospital Basel (coordinating centre for this pilot study).

Samples

Large quantities of virus suspension were needed for the EQA. For this reason, it was decided to culture the virus to generate enough material. Vero76 cells were grown in Dulbecco's modified Eagle's medium (DMEM; 10% fetal bovine serum; 1% glutamine) in flat-bottom 96-well plates (ThermoFischer Scientific, MA, USA). 100 µL of SARS-CoV-2 positive naso-oropharyngeal fluids were added and cells were incubated for 48 hours at 37°C. Cell culture supernatants were harvested, and SARS-CoV-2

RNA was quantified using the laboratory-developed Basel-SCoV2-112bp NAT, as described previously (9), targeting specific viral sequences of the spike glycoprotein *S* gene.

A total of 10 samples (named NGS1-10) of cell culture supernatants were frozen and shipped on dry ice to participating laboratories. The viral isolates originated from routine diagnostic samples from Clinical Virology, University Hospital Basel, reflecting diverse epidemiological backgrounds. The cell culture supernatants used contained a range of viral loads of SARS-CoV-2, reflecting viral loads typically observed in routine diagnostics of acutely ill COVID-19 patients (**see web-only Supplementary Table S1**). To ensure that no changes occurred during culture, both primary material and cell culture supernatant were sequenced and compared; the resulting sequences were identical (results not shown).

Assessment of variant calling

SNPs, as compared to the reference Wuhan-Hu-1, were assessed as reported (usually in form of a list of variants). In order to compare across centres and samples, a score was developed. As there is no “correct solution” to compare results against, a majority consensus approach was chosen, i.e. a SNP/indel was considered correct if the majority of labs detected it (ignoring missing data). If the correct base was called, a score of 1 was given per site. Incorrect base calls were scored with -1, respectively, missing data received 0. If an ambiguous base was called where a true SNP occurred, and the correct base was included in the ambiguity code (IUPAC), a score of 0.5 was given. Otherwise reported ambiguous sites were not counted as SNPs. In case of deletions that were present but not reported, we chose to set the score to -1, given that centres were instructed to report deletions and

failure to report could be an artefact of the bioinformatics pipeline. The score was finally normalised per sample by the number of correct SNPs.

Assessment of lineage and cluster assignment

The "correct answer" was again assumed to be the majority consensus. Clusters were re-labelled to unify the nomenclature and compare laboratories. We did not provide a strict definition of a cluster, but allowed laboratories to determine clusters based on internal criteria. In addition, no classical epidemiological metadata were provided, to help with potential interpretations.

Results

Genome depth, coverage and assembly

Mean read depth per centre ranged from 313x to 37,172x which reflects a >100-fold difference across centres. However, this was mostly driven by centre 14, which sequenced to extremely high read depth (**figure 1A, Supplementary Table S2**). Centres 7 and 9 are on the lower end of the spectrum (mean depth of 325x ±275 (SD) and 313x ±132, respectively), whereas all other labs usually sequenced to a mean depth between 1000x and 8000x.

The majority of samples could be assembled to a consensus genome by all centres with the exception of NGS8 for which assembly failed partially for centre 7 and completely failed for centre 9 as seen by the percentage of missing data shown in **figure 1B** (numeric values in web-only **Supplementary Table S3**).

SNPs and Indels

Variants have been assessed as reported and are displayed in **Supplementary Figure S1A-J** as a dot plot indicating presence and absence of the variant. Some centres have reported mixed sites using ambiguous codes while others did not. Moreover, not all centres reported deletions. Whether these have been correctly called in the consensus genome was therefore checked for each variation and, if present, specifically marked in **Supplementary Figure S1**. Additionally, Supplementary Table S5 lists the number of correct, wrong and missing SNP calls, respectively, for each sample and lab.

A variant calling score was developed in order to quantify and compare the variant calling per sample and lab (see methods). The results are shown in **figure 1C** (numerical value in **Supplementary Table S4**), with average score per sample across all centres (row marked with ø) also shown as a measure of congruence across laboratories. As expected, samples with a higher proportion of missing data produced a lower score if the affected regions harboured many variations (e.g., NGS3 by centre 7 which had a coverage of 91%). Samples NGS7, -9, and -10 had many deletions, and labs not reporting these deletions received a corresponding lower score. NGS8, however, was a sample with which many centres had problems. Many labs reported missing data for variant loci. Additionally, incorrect base calls were made, in particular by centre 15 (**Supplementary Figure S1H**). A combination of several of these factors can in turn result in a lower mean score for a centre (e.g. centre 7 with an average score of 0.75, Supplementary Table S4).

Lineage assignment

Correct lineage assessment is of course dependent on correct SNP calling and sufficient coverage across the genome. The majority of centres assigned all samples to the correct lineage (**table 2**). Two centres with the lowest mean depth failed in correctly assigning the lineage of one sample, NGS8 (B.1.177; **Supplementary Table S2**). Centre 7, which provided a 57% complete genome (mean read depth 39x), could assign the sample to lineage B. Rather surprisingly, the laboratory with the by far highest depth, centre 14, assigned the lineages of two samples incorrectly: NGS7 and -9 were both only assigned as lineages A, as opposed to the more accurate “correct solution” of A.27. This was due to an outdated version of pangolin.

246 Cluster identification

247 Almost all centres reported the same clusters (**table 3**). Samples NGS2 and NGS5 formed
248 one cluster (B); NGS3, NGS6, and NGS8 formed the second cluster (C), and NGS7 and NGS9
249 formed the third cluster (E).

250

251 The low coverage for sample NGS8 was a challenge for the two previously mentioned
252 centres 7 and 9. However, centre 7 reported a presumed allocation into the correct cluster
253 using the partial genome (highlighted in green in **table 3**). Centre 9 could not identify the
254 cluster due to the unsuccessful sequencing (9x mean depth, **Supplementary Table S2,**
255 highlighted in red). This resulted in a too small cluster.

256

257 Centre 12 had difficulties with two samples (NGS1 and -4) and allocated them incorrectly to
258 cluster B (together with NGS2 and -5, highlighted in yellow). This was despite them falling
259 into different Pango lineages (**table 2**). Centre 14 incorrectly assigned NGS1 and NGS4 to a
260 separate cluster (highlighted in blue), again despite differing Pango lineage assignments.
261 However, the other clusters were correctly assigned by both laboratories.

Discussion

Impact of methodological choices

Given that laboratories had free choice over their experimental as well as analytical protocols, disentangling the individual effects of these differences is impossible. A known factor to influence sequencing success is viral load. For example, NGS8, while having a comparable viral load to NGS9 and -10 (Ct of 28.4 and 28.1, respectively), was on the lower end of the spectrum (Ct value of 28, **Supplementary Table S1**). This could be why many centres had problems with this sample.

When grouping the sequencing method roughly into Illumina single-end vs Illumina paired-end vs Oxford Nanopore Technologies (ONT), a platform-related effect does not seem to have occurred (**Supplementary Figure S2**). In fact, centres 7 and 8 had a very similar sequencing setup, with the exception of their analysis pipeline (**table 1**). Centre 8 however was able to sequence to a greater depth and was therefore better able to perform accurate genomic analyses as they achieved overall higher coverage across the genome. Moreover, the small genome of SARS-CoV-2 and lack of long repeat regions allows the use of short reads or single-end sequencing which for other pathogen WGS would be more problematic.

Mean depth had an effect only insofar as too low depth leads to too much missing data. Once a sufficient read depth has been achieved, there was no further clear correlation between the score of variant calling and depth (**Supplementary Figure S3**). In general, depth across the genome can be very uneven and average depth as a measure does not fully take this into account. Technically, read depths between 100-200x can be enough for

genotyping. For example, samples NGS2 and -5 for centre 7 have 191x and 131x, respectively, as well as a low amount of missing data, and a high variant calling score (**figure 1**). However, when coverage is uneven, missing data can still be an issue even at higher average depth (e.g., NGS10 for centre7 at 246x, **figure 1, Supplementary table S2**). For accurately genotyping SARS-CoV-2, it is necessary to capture the entirety of the genome and not just some areas (even of biologically important such as the *S* gene) as the software used to determine the lineage built its models based on whole genome diversity (the pangoLEARN algorithm within pangolin) (8). It is therefore important to strive for the best coverage across the genome (i.e., a low amount of missing data) and "sufficient read depth", as mentioned above, is therefore a function of this. More even coverage in amplicon-based sequencing can for example be achieved by balancing primer sets.

Instead of average depth, other factors such as variant reporting capacity, mapping quality as well as interpretation of data play a larger role. This is an important point for diagnostic labs with respect to operational costs. The importance of this was highlighted by centre 14 which sequenced to the by far highest depth but had nevertheless difficulties with lineage and cluster assignment despite very good variant calling. Upon receiving a preliminary report, centre 14 re-examined their analysis pipeline and found they had used an outdated Pangolin and pangoLEARN version. The Pango lineage nomenclature is dynamic, meaning that nomenclature system develops as SARS-CoV-2 evolves, and lineage definitions and names can change over time (8). The pilot EQA provided here valuable feedback for the respective centre to improve its workflows.

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309 The cluster assignment, on the other hand, highlighted another challenge for the
310 development of any EQA: communication and interpretation. The majority of other centres
311 determined a cluster as a putative transmission cluster that differ between 0 amd maximally
312 2 SNPs (thresholds slightly vary, **Supplemental Methods**). Two centres had difficulties,
313 which could be resolved upon feedback. Centre 12 had interpreted the terminology
314 "cluster" differently and reported instead the Nextclade assignment (10); Centre 14 in turn
315 deemed samples NGS1 and NGS4 to belong to a single cluster. While they share a common
316 ancestor, most other labs deemed them sufficiently different to assign them to two
317 separate clusters. In fact, they differ in 27 SNPs, whereas the other true clusters (B, C, E in
318 **table 3**) had 0-1 SNPs between genomes. This highlights that there is a certain element of
319 subjectivity in data interpretation when lacking clear definitions as well as the need to
320 clarify the objective of the task (in this case the assessment of transmission clusters rather
321 than simply related sequences in a phylogenetic tree).
322
323 An important factor for routine sequencing is cost. In general, the amplicon-based protocols
324 used in this study consist of a reverse transcription step, an amplification step, the library
325 preparation, and the sequencing. As the first two steps are mostly the same for different
326 sequencing technologies, cost is driven mainly by the library preparation and sequencing
327 itself. Here, Oxford Nanopore (ONT) allows faster data generation due to real-time base
328 calling, while sequencing on an Illumina machine typically takes a little bit more than a day
329 (11). Cost-wise, the price per sample will decrease with increasing throughput. But the many
330 library preparation kits available as well as the wide range of sequencing machines used
331 here (**table 1**) make a comparison between the centres difficult.
332

All protocols used by the participating centres in this EQA used amplicon-based sequencing, and primer bias can have an influence on sequencing accuracy. Here, primer sets vary between labs (**table 1**). For the Artic v3 primers (which are public), we find no apparent bias in the data reported here compared to the other primer panels. However, centres 7 and 8 which used the same primer panel but did not detect the variant G21255C in samples NGS3, -6 and -8 (**Supplementary figure 1C, F, H**). This SNP is present in almost all representatives of lineage B.1.177 (12). Whether this failure in detection is truly due to a primer bias cannot be conclusively answered though, as commercial primer sequences are often not public. A possibility to deal with this issue bioinformatically is to trim primer sequences prior to assembly. Nevertheless, primer bias is a real issue if it leads to dropouts. Fortunately, it is actively monitored by the community. For example, dropouts of the Artic v3 panel have been reported especially for Beta and Delta variants. For this reason, a new primer panel has been developed to avoid high frequency variant sites in the newer lineages (13).

Factors not assessed in this pilot EQA

This pilot EQA focussed on reporting findings relating to consensus genome sequences, but did not include minority variant reporting. Centre 15 reported issues with contamination for sample NGS8, yet lineage and cluster assignment were successful as the key sites were not affected. However, some contamination spilled over into the consensus genome as evidenced by a number of wrong variant calls (**Supplementary Figure S1H**). Similarly, some labs reported mixed loci as SNPs in their report, although we were mostly interested in fixed changes. Differentiating between contamination from true, albeit rare, mixed infections or possible in-host evolution can be very difficult, especially in a clinical setting with high sample throughput. Assessment of contamination and analysis of minority variants would

allow the provision of more detailed feedback to the laboratories. Contamination, for example, would likely be an isolated event for a centre, resulting in mixed sites, while true mixture would be prevalent across all centres. At the same time, it would offer an interesting analytical challenge, in particular if samples with true mixed infections were sent to participants.

Conclusion and lessons learnt

The first ESGMD-SSM pilot EQA of SARS-CoV-2 sequencing was overall a success. Most centres generated whole genome sequences and correctly identified all lineages and clusters. Additionally, there was a general consensus regarding the majority of called SNPs, despite the strong effect that missing data and unreported deletions (although present in the data) had on the scores of some. This suggests an overall high quality in each participating centre. The standardised reporting of important variations in the genome should be the focus of improvement for some bioinformatic pipelines. The most critical aspect was coverage across the genome, which correlated with correct lineage and cluster assignment.

For a follow-up EQA, the variables and format of the variables to document have to be more clearly defined. Moreover, minority variants should be included to some degree from samples with mixed infections. Information on primer sets for amplicon-based methods should be carefully recorded, especially in light of new virus lineages. Instead of culture supernatants it might also be of interest to include primary patient samples diluted in clinical collection matrix as well as an empty control. Finally, to trigger a discussion on cluster definition, samples with high similarity but 2-5 SNP difference could also be included.

The COVID-19 pandemic required a rapid global laboratory response involving the development and roll-out of new diagnostic assays and diagnostic platforms on an unprecedented scale. In response to the emergence and spread of virus variants of concern, WGS is increasingly being utilised, not only for surveillance but also for diagnostic purposes, thus necessitating the rapid deployment and sharing of quality assurance schemes. This EQA pilot provides a proof-of-feasibility for development and operationalisation of an EQA for WGS in a pandemic context and lessons learnt from its design, delivery and results should inform future pandemic preparedness.

Conflict and interest statement & acknowledgements.

The authors have no conflict of interest to declare regarding SARS-CoV-2 diagnostics. Costs of preparing the EQA and shipping costs were borne by the Clinical Bacteriology and Mycology group of the University Hospital of Basel and the Swiss Society of Microbiology (A Egli), whereas sequencing costs were supported by each participating laboratory. This work was in part supported by B-FAST (Fkz 01KX2021) in the framework of the National Research Network of University Medical Centers on COVID-19 (Nationales Forschungsnetzwerk der Universitätsmedizin zu Covid-19), Federal Ministry of Education and Research of Germany (Bundesministerium für Bildung und Forschung, BMBF). This pilot EQA has been initiated by Prof A Egli (current head of the “Coordinated Clinical Microbiology” section of the Swiss

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Society of Microbiology (SSM) and by Prof. G Greub the current chairman of European Study Group on Genomics and Molecular Diagnosis (ESGMD).

References

1. ECDC. Sequencing of SARS-CoV-2: first update [Internet]. 2021 Jan [cited 2021 Jun 17]. Available from: https://www.ecdc.europa.eu/en/publications-data/sequencing-sars-cov-2
2. Karmarkar E, Blanco I, Amornkul P, Dubois A, Deng X, Moonan PK, et al. Timely Intervention and Control of a Novel Coronavirus (COVID-19) Outbreak at a Large Skilled Nursing Facility-San Francisco, California, 2020. Infection Control and Hospital Epidemiology. 2020;
3. Pillay S, Giandhari J, Tegally H, Wilkinson E, Chimukangara B, Lessells R, et al. Whole genome sequencing of sars-cov-2: Adapting illumina protocols for quick and accurate outbreak investigation during a pandemic. Genes. 2020;11(8).
4. Stange M, Marii A, Roloff T, Seth-Smith HMB, Schweitzer M, Brunner M, et al. SARS-CoV-2 outbreak in a tri-national urban area is dominated by a B.1 lineage variant linked to a mass gathering event. PLoS Pathogens. 2021;17(3).
5. Brüningk SC, Klatt J, Stange M, Mari A, Brunner M, Roloff T-C, et al. Determinants of SARS-CoV-2 transmission to guide vaccination strategy in a city. medRxiv. 2020.
6. Junier T, Huber M, Schmutz S, Kufner V, Zagordi O, Neuenschwander S, et al. Viral metagenomics in the clinical realm: Lessons learned from a swiss-wide ring trial. Genes. 2019;10(9).
7. Dylus D, Pillonel T, Opota O, Wüthrich D, Seth-Smith HMB, Egli A, et al. NGS-Based S. aureus Typing and Outbreak Analysis in Clinical Microbiology Laboratories: Lessons Learned From a Swiss-Wide Proficiency Test. Frontiers in Microbiology. 2020;11.
8. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nature Microbiology. 2020;5(11).
9. Leuzinger K, Gosert R, Søgaard KK, Naegele K, Bielicki J, Roloff T, et al. Epidemiology and precision of SARS-CoV-2 detection following lockdown and relaxation measures. Journal of Medical Virology. 2021;93(4).
10. Aksamentov I, Neher R. Nextclade. https://github.com/nextstrain/nextclade. 2020.
11. Hourdel V, Kwasiborski A, Balière C, Matheus S, Batéjat CF, Manuguerra JC, et al. Rapid Genomic Characterization of SARS-CoV-2 by Direct Amplicon-Based Sequencing Through Comparison of MinION and Illumina iSeq100TM System. Frontiers in Microbiology. 2020;11.
12. Hodcroft EB, Zuber M, Nadeau S, Vaughan TG, Crawford KHD, Althaus CL, et al. Spread of a SARS-CoV-2 variant through Europe in the summer of 2020. Nature. 2021;595(7869).

13. Davis JJ, Long SW, Christensen PA, Olsen RJ, Olson R, Shukla M, et al. Analysis of the ARTIC version 3 and version 4 SARS-CoV-2 primers and their impact on the detection of the G142D amino acid substitution in the spike protein. bioRxiv. 2021;
14. SmaltAlign. https://github.com/medvir/SmaltAlign.
15. Loman N, Rambaut A. nCoV-2019 novel coronavirus bioinformatics protocol. https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html.
16. GENCOV. https://github.com/metagenlab/GENCOV.
17. Maier W, Bray S, van den Beek M, Bouvier D, Coraor N, Miladi M, et al. Freely accessible ready to use global infrastructure for SARS-CoV-2 monitoring. bioRxiv. 2021;
18. Jalili V, Afgan E, Gu Q, Clements D, Blankenberg D, Goecks J, et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update. Nucleic Acids Research. 2021;48(W1).
19. MACOVID. https://github.com/MUMC-MEDMIC/MACOVID.
20. Oude Munnink BB, Nieuwenhuijse DF, Stein M, O'Toole Á, Haverkate M, Mollers M, et al. Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands. Nature Medicine. 2020;26(9).
21. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25(3).
22. Wolters F, Coolen JPM, Tostmann A, van Groningen LFJ, Bleeker-Rovers CP, Tan ECTH, et al. Novel SARS-CoV-2 whole-genome sequencing technique using reverse complement PCR enables fast and accurate outbreak analysis. bioRxiv. 2020.
23. Easyseq. https://github.com/JordyCoolen/easyseq_covid19.
24. SusCovONT. https://github.com/marithetland/susCovONT.
25. Coolen JPM, Wolters F, Tostmann A, van Groningen LFJ, Bleeker-Rovers CP, Tan ECTH, et al. SARS-CoV-2 whole-genome sequencing using reverse complement PCR: For easy, fast and accurate outbreak and variant analysis. Journal of Clinical Virology. 2021;144.
26. Health 2030 Genome Center SARS-CoV2 pipeline. https://github.com/health2030genomecenter/SARS-CoV-2_pipeline.
27. Grubaugh ND, Gangavarapu K, Quick J, Matteson NL, de Jesus JG, Main BJ, et al. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. Genome Biology. 2019;20(1).
28. Pagès H, Aboyoun P, Gentleman R, DebRoy S. Biostrings: Efficient manipulation of biological strings. R package version 2.46.0. R package version 2.46.0. 2017.
29. Mari A, Roloff T, Stange M, Søgaard KK, Asllanaj E, Tauriello G, et al. Global Genomic Analysis of SARS-CoV-2 RNA Dependent RNA Polymerase Evolution and Antiviral Drug Resistance. Microorganisms. 2021 May 19;9(5).
30. Mari A. COVGAP. https://github.com/appliedmicrobiologyresearch/covgap.

Figure Captions

Figure 1: A) Mean read depth per sample (x axis) and centre (y axis). Colours have been scaled for high resolution for values between 0 and 10,000; values bigger than this are displayed in the same colour. B) Percentage of Ns in the genome per sample (x axis) and centre (y axis). C) Score for variant detection per sample (x axis) and centre (y axis) as well as mean score for each centre across all samples and mean score for each sample across centres (ø). The numerical values underlying each plot can be found in the Supplementary Table S2-4.

Tables

Centre	Primer panel	Sequencing technology	Bioinformatics	References
1	ARTIC nCoV-2019 v3	Illumina MiSeq, 150pb SE	SmaltAlign	(14)
2	ARTIC nCoV-2019 v3	Nanopore	Artic bioinfo pipeline v1.1.3	(15)
3	ARTIC nCoV-2019 v3	Illumina MiSeq, 150pb PE	virSEAK pipeline (JSI Medical Systems)	
4	CleanPlex SARS-CoV-2 (Paragon Genomics)	Illumina MiSeq, 150pb PE	GENCOV	(14)
5	ARTIC nCoV-2019 v3	Illumina MiSeq, 150pb PE	custom Galaxy pipeline	(17,18)
6	custom	Nanopore	MACOVID pipeline	(19,20)
7	EasySeq RC-PCR SARS-CoV-2 (NimaGen)	Illumina, MiniSeq, 150bp PE	custom pipeline	(21,22)
8	EasySeq RC-PCR SARS-CoV-2 (NimaGen)	Illumina, MiniSeq, 150bp PE	EasySeq pipeline	(23)
9	Midnight primer panel (IDT)	Nanopore	Artic bioinfo pipeline	(15)
10	ARTIC nCoV-2019 v3	Nanopore	Artic bioinfo pipeline	(15,21)
11	ARTIC nCoV-2019 v3	Nanopore	SusCovONT	(24)
12	QIAseq SARS-CoV-2 Primer Panel (QIAGEN)	Illumina MiniSeq, 150pb PE	Illumina BaseSpace DRAGEN COVID Lineage	
13	Illumina COVIDSeq Test	Illumina, NovaSeq, 50bp PE	Health 2030 Genome Center in Geneva pipeline	(26)
14	Illumina COVIDSeq Test	Illumina, NovaSeq, 150bp PE	custom pipeline	(27,28)
15	ARTIC nCoV-2019 v3	Illumina, NextSeq, 150bp PE	COVGAP	(4,29,30)

Table 1: Summary of the methods used by the participating centres. A detailed method description by each centre can be found in the supplementary material.

Centre	NGS1	NGS2	NGS3	NGS4	NGS5	NGS6	NGS7	NGS8	NGS9	NGS10
1	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
2	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
3	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
4	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
5	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
6	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
7	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B	A.27	B.1.1.7
8	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
9	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	N/A	A.27	B.1.1.7
10	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
11	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
12	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
13	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7
14	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A	B.1.177	A	B.1.1.7
15	B.1.416.1	B.1.36.17	B.1.177	B.1.258	B.1.36.17	B.1.177	A.27	B.1.177	A.27	B.1.1.7

502 Table 2: Pango lineage assignments. Red highlights a case where lineage assignment was

503 impossible. Blue highlights cases discussed in more detail in the main text.

504

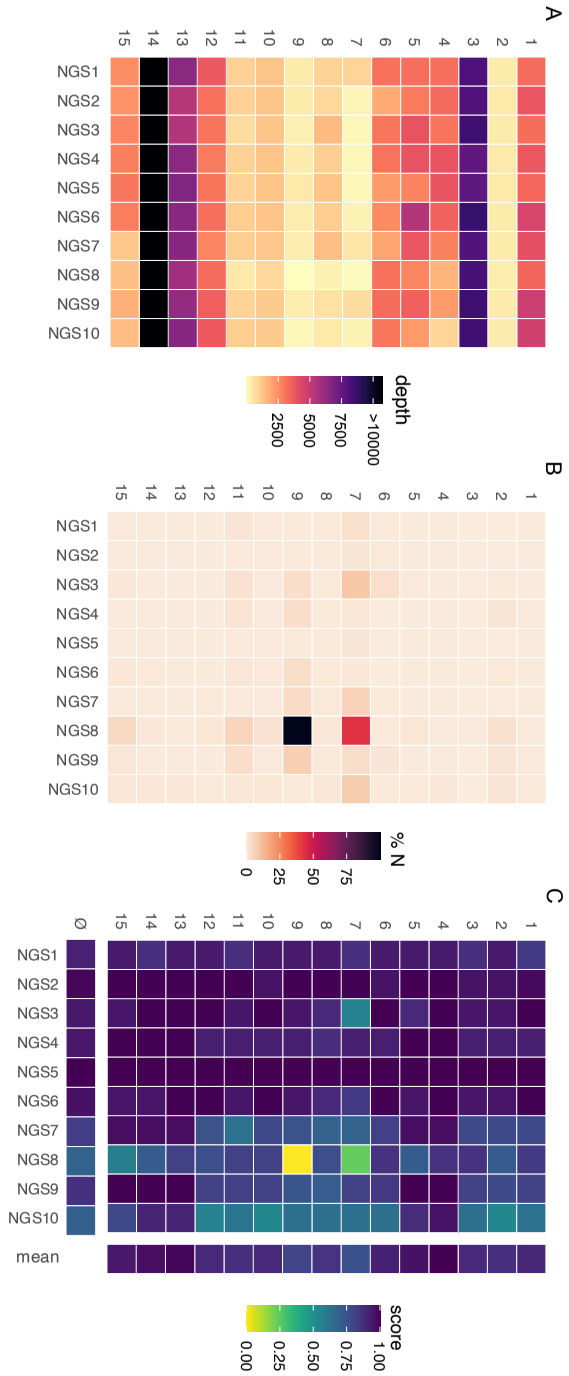
Centre	NGS1	NGS2	NGS3	NGS4	NGS5	NGS6	NGS7	NGS8	NGS9	NGS10
1	A	B	C	D	B	C	E	C	E	F
2	A	B	C	D	B	C	E	C	E	F
3	A	B	C	D	B	C	E	C	E	F
4	A	B	C	D	B	C	E	C	E	F
5	A	B	C	D	B	C	E	C	E	F
6	A	B	C	D	B	C	E	C	E	F
7	A	B	C	D	B	C	E	C*	E	F
8	A	B	C	D	B	C	E	C	E	F
9	A	B	C	D	B	C	E	N/A	E	F
10	A	B	C	D	B	C	E	C	E	F
11	A	B	C	D	B	C	E	C	E	F
12	B	B	C	B	B	C	E	C	E	F
13	A	B	C	D	B	C	E	C	E	F
14	A	B	C	A	B	C	E	C	E	F
15	A	B	C	D	B	C	E	C	E	F

505 Table 3: Cluster assignments. Red highlights a case where cluster assignment was

506 impossible. Green, yellow and blue highlight discrepant cases discussed in more detail in the

507 main text. The * marks that the centre reported an assumed cluster assignment based on a

508 partial genome.



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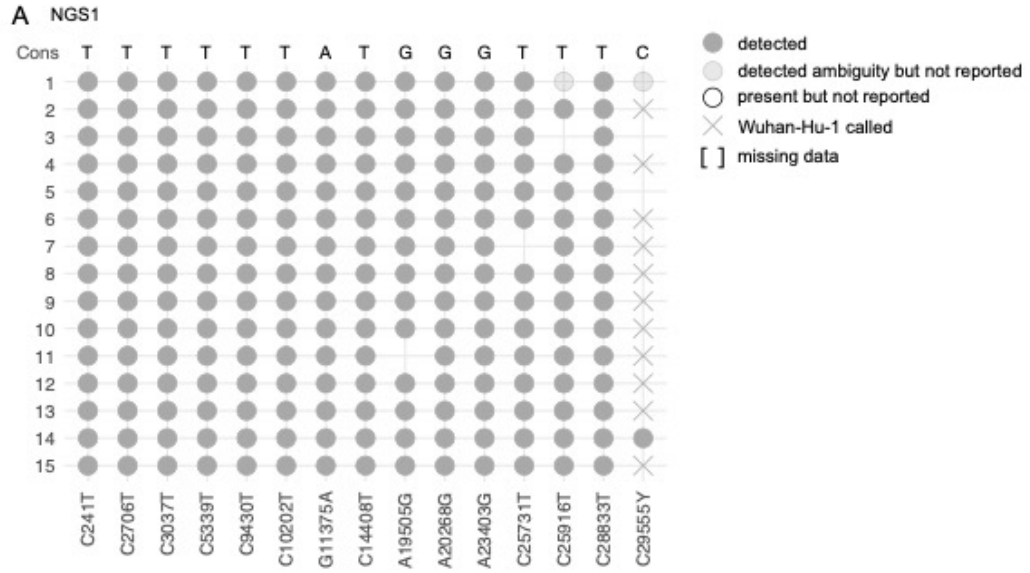
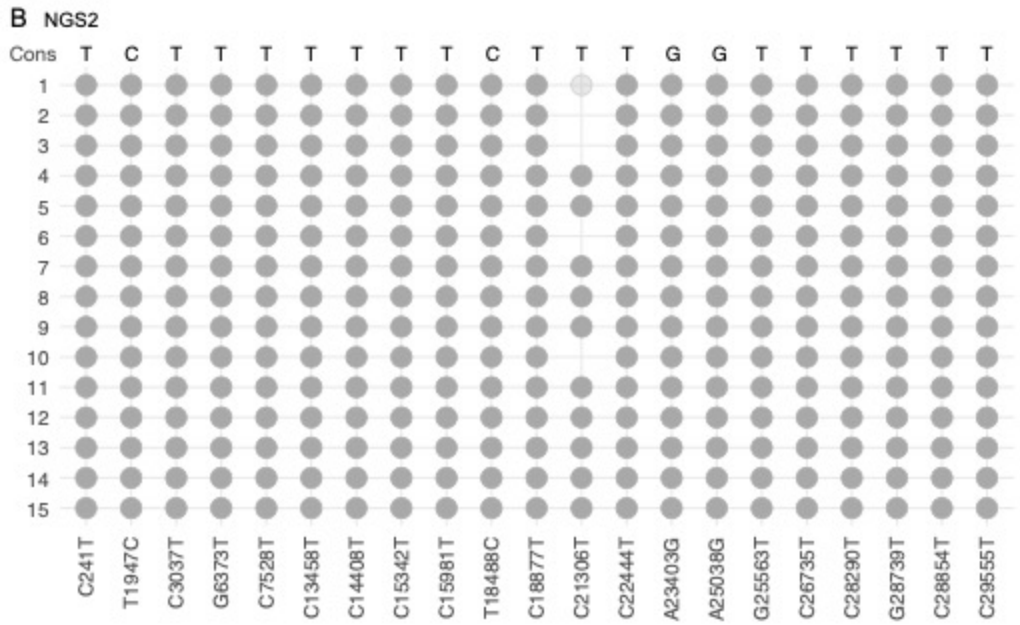
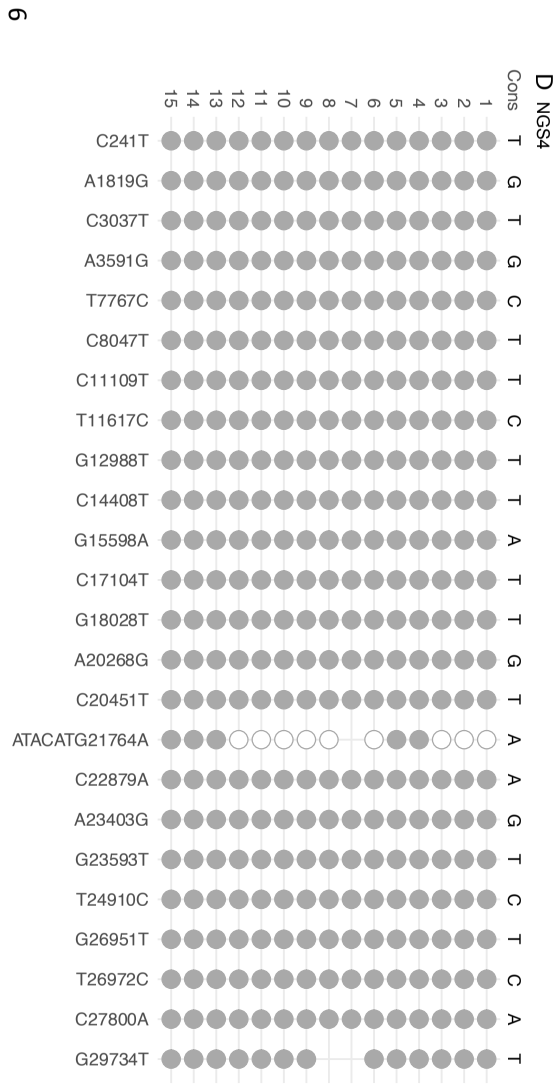
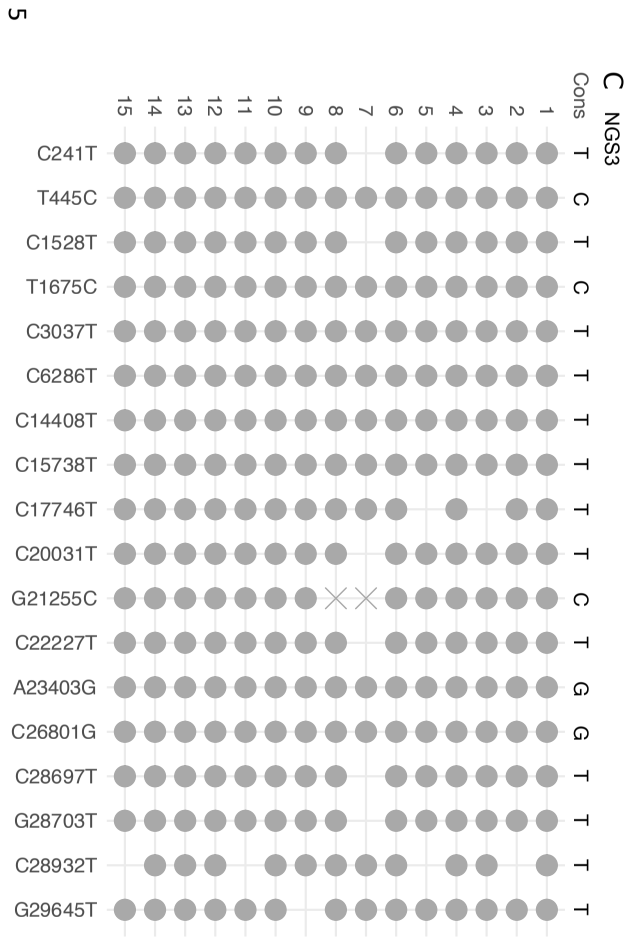
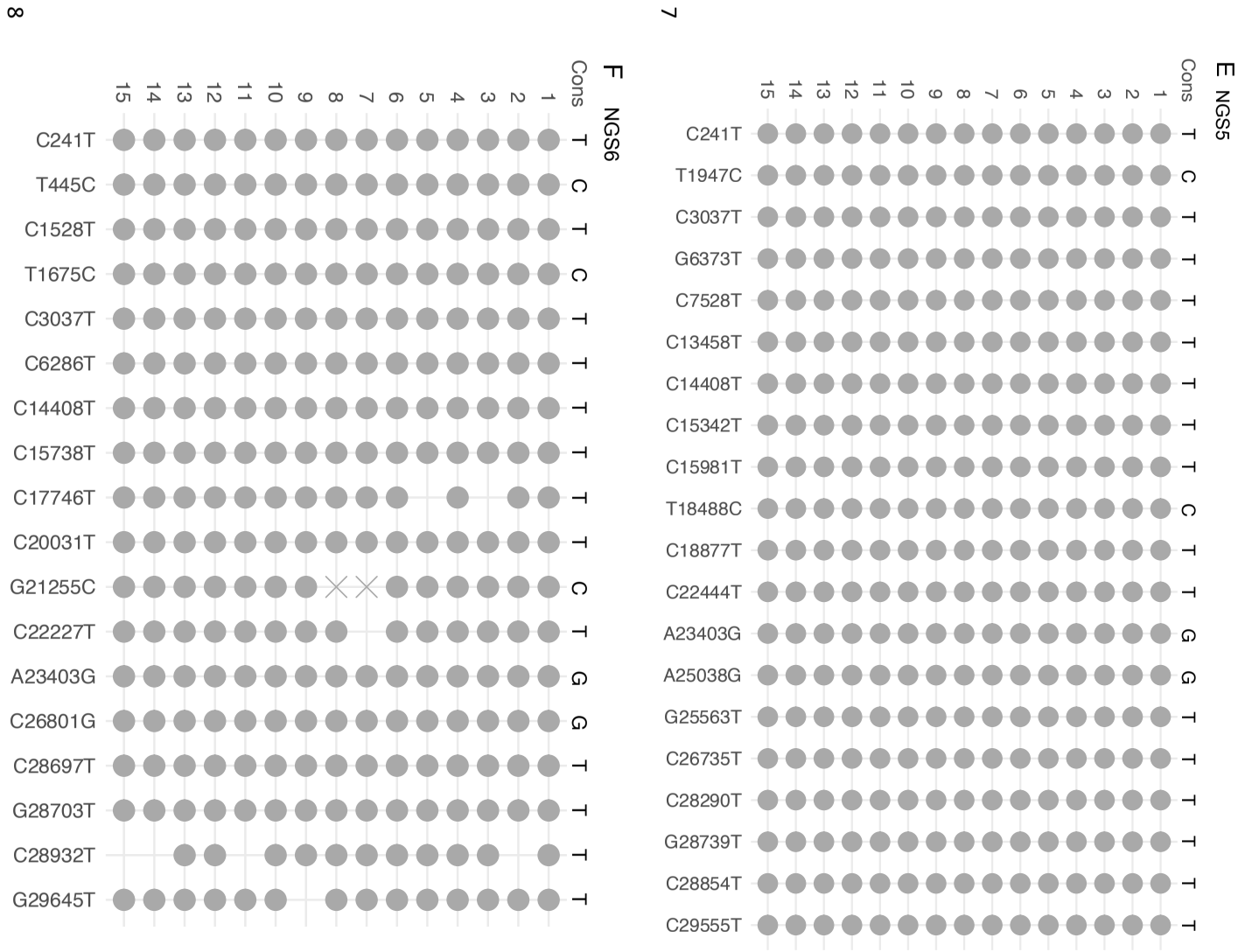
Supplemental Figures

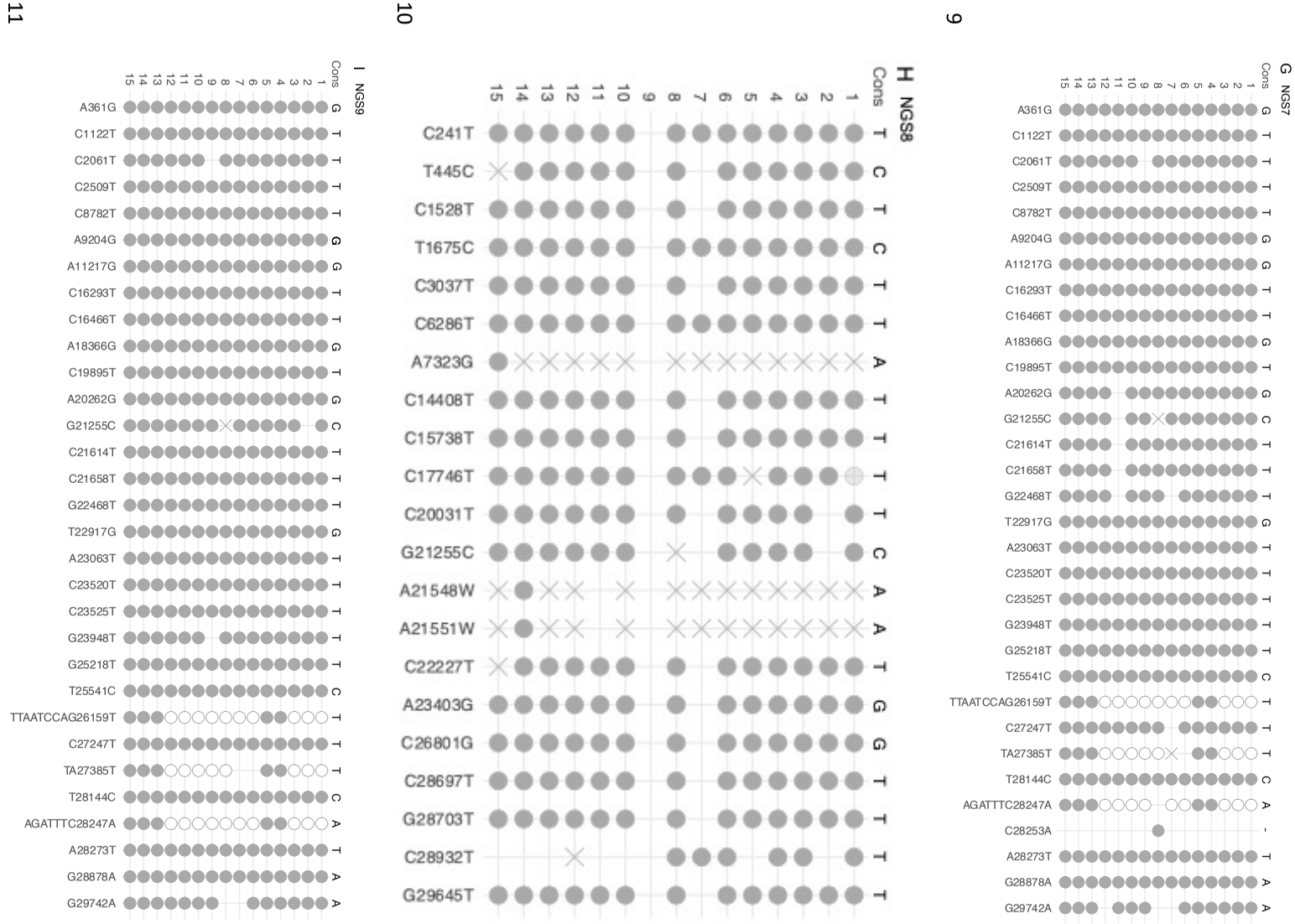


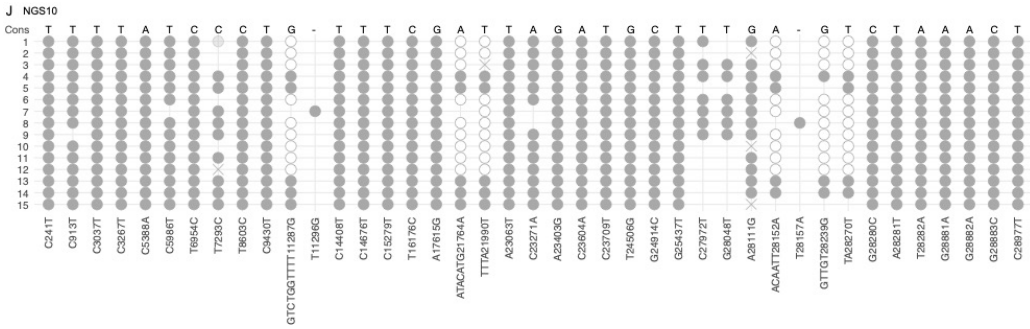




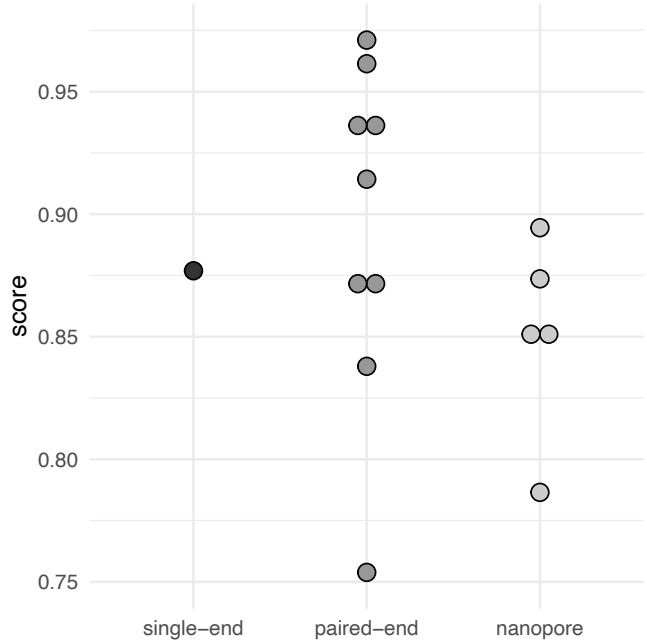




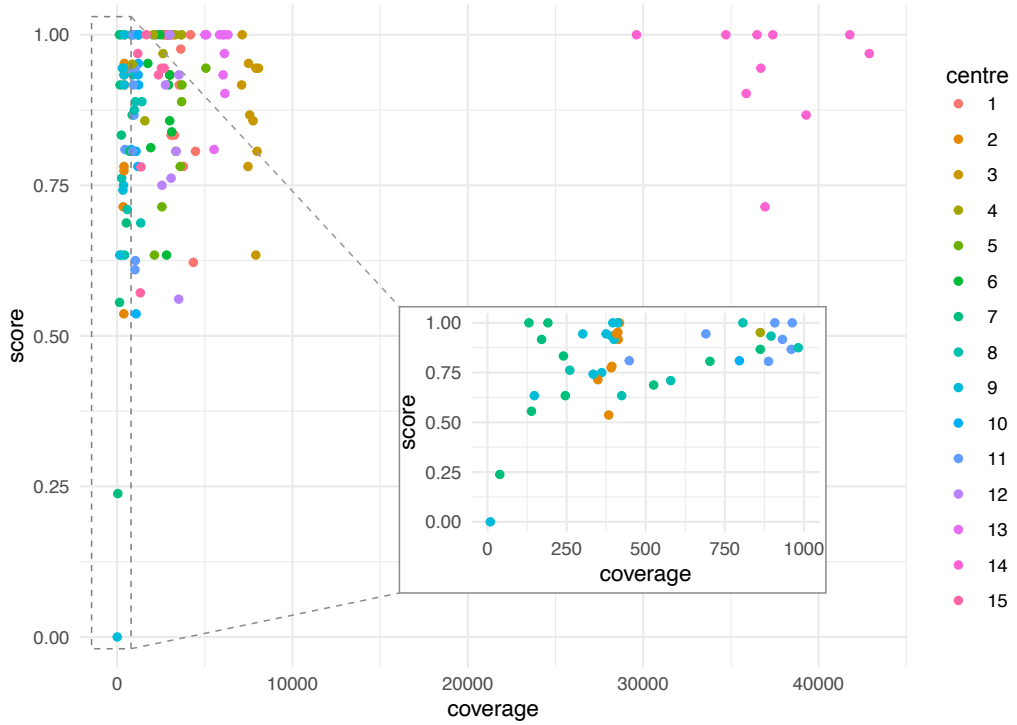




Suppl. figure S1: A-J Presence and absence of SNPs and Indels per sample. On the x-axis, all variations that were specifically reported by the centres are listed. On the y-axis are the centres. A dark grey filled circle means the respective SNP was reported. No symbol means the genome sequence has an N at that position. A cross indicates that instead of the SNP, the reference position was called; this can either be because the SNP is not true or because the base call is wrong. Additionally, sometimes ambiguous sites were reported as SNPs or are present in the consensus genome at the position of a reported SNP. If such a position was found in the sequence (but not reported) a less opaque filled circle is shown. Lastly, some centres did not report deletions. If these non-reported deletions were nevertheless present in the data, they are indicated with a white-filled circle.



Suppl. figure S2: Mean variant calling score per lab depending on the sequencing methods used.



Suppl. figure S3: Variant calling score for each sample and centre depending on the mean coverage.

Supplemental Methods

Centre 1

SARS-CoV-2 whole-genome sequencing was performed according to the nCoV-2019 sequencing protocol v3 (LoCost) V.3 (1). Briefly, total nucleic acids were extracted followed by reverse transcription with random hexamers using LunaScript RT SuperMix Kit (NEB). The generated cDNA was used as input for two pools of overlapping PCR reactions (ca. 400nt each) spanning the viral genome using Q5 Hot Start High-Fidelity 2X Master Mix (NEB). Amplicons were pooled per patient before NexteraXT library preparation and sequencing on an Illumina MiSeq for 1 × 151 cycles. To generate SARS-CoV-2 consensus sequences, reads were iteratively aligned using SmaltAlign (2). Clusters were determined manually based on phylogenetic analysis.

Centre 2

A typical Nanopore sequencing library consisted of the pooling of PCR amplicons generated according to the ARTIC v3 protocol (3), which generates 400 bp amplicons that overlap by approximately 20 bp. Library preparation was performed with SQK-LSK109 (Oxford Nanopore Technologies, Oxford, UK) according to the ONT "PCR tiling of COVID-19 virus" (version: PTC_9096_v109_revE_06Feb2020, last update: 26/03/2020). Reagents, quality control and flow cell preparation were done as described previously (4,5). ONT sequencing was performed on a GridION X5 instrument (Oxford Nanopore Technologies) with real-time basecalling enabled (ont-guppy-for-gridion v.4.2.3; fast basecalling mode). Sequencing runs

were terminated after production of at least 100,000 reads per sample. Bioinformatic analyses followed the workflow described (3) using artic version 1.1.3. Consensus sequences were generated using medaka (6) and bcftools (7). For cluster determination, the consensus sequences were aligned using muscle (v3.8.1551, options -maxiters 1 -diags), and the number of nucleotide differences between each sequence pair was calculated with R (version #.6.0) using the R libraries seqinr and dplyr. Cluster definition was set as no SNV difference between any sequences in a given cluster.

Centre 3

The RNA of the samples was extracted with the Maxwell RSC Viral TNA kit and tested with our inhouse-house SARS-CoV-2 assay. The reverse transcription was done with the LunaScript RT Super Mix (NEB), followed by amplification of the SARS-CoV-2 genome according to the amplicon sequencing strategy of the ARCTIC protocol with re-balanced V.3 primers. Library construction was performed with the Illumina DNA Prep (M) kit according to the manufacturer's instructions. After quantification, an equal amount of each library was pooled and sequenced on an Illumina MiSeq with 300 cycles and v2 chemistry. The bioinformatics analysis was done with the virSEAK pipeline (v2.0.11; JSI). The discrimination into the different clusters was done manually according to the designated Pango lineage.

Centre 4

RNA from nasopharyngeal or mouth swabs collected in COPAN UTM™ liquid (3.5 ml) were extracted on a MagNA Pure 96 instrument (Roche, Basel, Switzerland). All samples were

processed with the CleanPlex SARS-CoV-2 15 Panel and CleanPlex Dual Indexed (Paragon Genomics #918011) according to manufacturer's protocol. PCR products were analyzed using a Fragment Analyzer, « Standard Sensitivity NGS » (AATI, ref. DNF-473), and DNA was quantified with Qubit Standard Sensitivity dsDNA kit (Invitrogen, ref. Q32853). All samples were sequenced using paired-end 2x150bp MiSeq Illumina protocol (San Diego, USA). Sequence reads were processed using GENCOV (8), a modified version of CoVpipe (9). Briefly, reads were filtered with fastp (10) and mapped on SARS-CoV-2 reference genome NC_045512.2 with bwa (11). Qualimap (12) was used to evaluate the alignment and primer sequences from CleanPlex® panel were trimmed with fgbio (13). Variant calling was performed with freebayes (14) (Parameters: --min-alternate-fraction 0.1 --min-coverage 10 --min-alternate-count 9). Putative variants were filtered with bcftools (15) based on mean mapping quality (MQM > 40), variant quality (QUAL >10) and an alternate frequency of at least 70%. The consensus sequence generated with bcftools was assigned to SARS-Cov-2 lineages with pangolin (16).

Centre 5

Whole genome sequencing. cDNA was produced from extracted RNA using random hexamer primers and Superscript III (ThermoFisher) followed by a PCR tiling the entire SARS-CoV-2 genome (ARTIC V3 primer sets; (17)). This produced 400 bp long, overlapping amplicons that were subsequently used to prepare the sequencing library. Briefly, the amplicons were cleaned with AMPure magnetic beads (Beckman Coulter). Afterwards the QIAseq FX DNA Library Kit (Qiagen) was used to prepare indexed paired end libraries for Illumina sequencing. Normalized and pooled sequencing libraries were denatured with 0.2 N

NaOH. This 8 pM library was sequenced on an Illumina MiSeq instrument using the 300-cycle MiSeq Reagent Kit v2.

Bioinformatics. The de-multiplexed raw reads were subjected to a custom Galaxy pipeline (18,19). The raw reads were pre-processed with fastp (v.0.20.1) (10) and mapped to the SARS-CoV-2 Wuhan-Hu-1 reference genome (Genbank: NC_045512) using BWA-MEM (v.0.7.17) (20). For datasets, which were produced with the ARTIC v3 protocol, primer sequences were trimmed with ivar trim (v1.9) (21). Variants (SNPs and INDELs) were called with the ultrasensitive variant caller LoFreq (v2.1.5) (22) demanding a minimum base quality of 30 and a coverage of at least 5-fold. Afterwards, the called variants were filtered based on a minimum variant frequency of 10 % and on the support of strand bias. The effects of the mutations were automatically annotated in the vcf files with SnpEff (v.4.3.1) (23). Finally, consensus sequences were constructed by bcftools (v.1.1.0) (24). Regions with low coverage >5x or variant frequencies between 30 and 70 % were masked with Ns. The variant frequencies (>10%) of the nucleotide substitutions of the respective samples were matched in a matrix and clusters were determined by hierarchical clusterin (ward.D2) using the R package hclust. The script is available on GitHub (25)and was implemented on usegalaxy.eu.

Centre 6

Sequencing of SARS-CoV-2-positive samples

Samples were stored at -80 degrees Celsius until RNA was isolated for sequencing. For RNA extraction, 90 µl of sample was mixed with 90 µl of Chemagic Viral Lysis Buffer (Perkin-Elmer), followed by extraction using the MagNA Pure 96 DNA and Viral NA Small Volume

Kit 96 (Roche, Germany) on the MagNA Pure 96 system (Roche, Germany), without the addition of an internal extraction control.

Sequencing was performed using the PCR tiling of SARS-CoV-2 virus with Native Barcoding Expansion 96 (EXP-NBD196) protocol (Version: PTCN_9103_v109_revH_13Jul2020) of Oxford Nanopore technologies, with minor modifications and using the primers previously published by Oude Munnink et al. (26). Briefly, the only modifications were extending the barcode and adaptor ligation steps up to 60 min and loading 48 samples per flow cell.

Bioinformatic analysis was performed using an in-house developed pipeline MACOVID that is based on Artic v1.1.3. In brief, short and obvious chimeric reads are filtered with Cutadapt v2.5. The filtered reads were mapped to the reference genome MN908947.3 with Minimap2 v2.17 and quality checked with “align_trim” function of Artic v1.1.3. Mapped reads were split per primer pool using Samtools v1.9 and a consensus was created per primer pool with Medaka v1.0.3. Variants were called using Medaka v1.0.3 and Longshot v0.4.1. Low coverage regions (<30x) were masked with “artic_make_depth_mask” function of Artic v1.1.3. A preconsensus was made with “artic_mask” and the final consensus sequence was made with bcftools v1.10.2. Documentation and source code are available from (27) under MIT license. The consensus sequences were used to construct a phylogenetic tree with the ncov pipeline v3 of nextstrain. Samples were considered to be part of the same cluster of there are <= 2 SNPs difference. Pangolin lineages were assigned were assigned using the Pangolin COVID-19 Lineage Assigner web application on https://pangolin.cog-uk.io/.

121 Centre 7

122 Nucleic acid was extracted from 200 ul sample and eluted in 100 ul buffer using a MagNa
123 Pure 96 instrument (Roche Diagnostics). Ten microliters extract was added to the RT-PCR
124 assay for SARS-CoV-2 E-gene detection as described by Corman et al. (28) and performed on
125 a CFX96 PCR instrument (Bio-Rad): 50˚C for 5 min, followed by 95˚C for 20 s and then 45
126 cycles of 95˚C for 15 s, 55˚C for 10 s, and 72˚C for 50 s.
127 Whole genome sequencing (WGS) was performed using the EasySeq RC-PCR SARS-CoV-2
128 WGS kit (NimaGen BV). A detailed description of the technology has recently been
129 described by Coolen et al, 2020 (29). Bidirectional sequencing of the SARS-CoV-2 amplicons
130 was performed using the MiniSeq platform (Illumina), with fastQ-formatted sequences
131 being extracted from the MiniSeq machine and processed further using different
132 bioinformatic tools. First, quality filtering of reads, including trimming of primer sequences,
133 was performed using Trimmomatic (version 3) with the following settings: LEADING:3;
134 TRAILING:3; SLINDINGWINDOW:4:15; HEADCROP:32; MINLEN:40. Then, reads were mapped
135 with Bowtie2 (version 2.3.4, settings --local --qc-filter --quiet) to the NC_045512.2 SARS-
136 CoV-2 reference strain and further analyzed using the default settings of Samtools (version
137 1.7). The sequence read depth was calculated using the IGV tool (version 2.3.98, settings: -w
138 1). Values of read depth obtained for each position (NTs or indels) for all samples were
139 filtered using 0.5 as a minimum frequency of SNPs relative to the total depth at this
140 position, so S/VNPs with frequency of <0.5 were ignored. Positions with a read depth of <10
141 reads were also ignored and implemented in sequences as gaps and filled with Ns. A list of
142 SNPs found compared to NC_045512.2 was generated after uploading the consensus
143 sequences to Nextclade (version 0.14.2) and downloading the resulting CSV file. Finally,
144 sequences with >=50% non-gap positions were used for building a phylogenetic tree.

Phylogenetic analysis of the data was done with Nextstrain (version 1.16.5) and a maximum likelihood tree was built with IQ-TREE (settings: -ninit 2 -n 2 -me 0.05 -nt 1). Results of the analysis were represented as Auspice v2 JSON files. Clusters were identified by having no more than three SNP difference.

Centre 8

DNA sequencing and analysis was performed similar to method described in (29). In short: cDNA-synthesis was performed using Multiscribe RT (Applied Biosystems, CA, USA). Whole genome sequencing (WGS) was performed using EasySeqTM RC-PCR SARS-CoV-2 version 2 (NimaGen, Nijmegen, The Netherlands) to construct an Illumina compatible sequence library. DNA sequencing was performed using 2x151 bp paired-end sequencing on a Illumina MiniSeq with a Mid-output sequence kit. Variant Calling and construction of the consensus sequence was performed using a custom designed easyseq pipeline (version 0.5.2) (30). To determine the lineage Pangolin (version 2.3.2) with pangoLEARN (version 2021-02-21) was used. Sequences were considered to belong to a cluster if they differ maximum 1 SNP from each other.

Centre 9

Extracted RNA was reverse trancribed using LunaScript RT (NEB), PCR amplicons were generated using IDT Midnight primers and Q5 High-Fidelity master mix (NEB). Transposase based fragmentation and barcode ligation was performed using the Ligation locost protocol (Oxford Nanopore Technologies).

Consensus fasta sequences were generated using the tools from the artic network (3). Read filtering was performed with guppyplex with the following paramters ` --skip-quality-check --min-length 900 --max-length 1600`. The output from guppyplex was used as input for the (nanopolish) artic minion pipeline, with `--normalise 200` as parameter. A custom scheme using primers of 1200bp was used (31).

Lineages were assigned using the command-line version (2.3.4) of pangolin (16). Clusters were identified with the command-line version of nextclade (0.14.1) with a threshold of less than 2 SNP difference. Input for both programs was the consensus fasta sequence generated by the artic minion pipeline.

Mean coverage was calculated with the command-line version (0.2.6) of mosdepth (32). The value under 'mean' for row 'total' was taken.

Centre 10

RNA was isolated using an easyMAG extractor following manufacturer's instructions for extraction of total nucleic acids from airways samples (BioMérieux, Marcy-l'Étoile, France). Detection of SARS-CoV-2 virus was performed using a validated qualitative RT-PCR detecting the SARS-CoV-2 virus E-gene based on a method published by Corman et al (28). Eluted RNA was reverse transcribed and PCR amplified according to the Artic Network v3 protocol using the ARTIC nCoV-2019 version 3 primer set with annealing temperature at 63 °C during PCR. The PCR products were sequenced on a GridION sequencer (Oxford Nanopore Technologies, Oxford, UK). The Medaka-pipeline by the ARTIC network (3) was used to generate consensus

sequences and call variant nucleotides relative to the reference sequence. Called variants were visualised in Geneious Prime (v2020.0.4) for validation and comparison. The consensus sequences were aligned using MAFFT and a phylogenetic tree using FastTree algorithm was generated to visualise the relatedness of the sequences in Geneious Prime. The criteria for samples being within an outbreak cluster was defined as sequences with < 3 SNPs differences.

Centre 11

RNA were extracted on a Biomek i7 automated workstation (Beckman Coulter) using their RNAdvanceViral kit (C63510) and protocol (and a Ct value from an in house Sarbeco-PCR provided). Further, we performed the Artic protocol v3 for PCR and library prep (1) using the ARTIC nCoV-2019 v3 primer panel from Integrated DNA technologies (Cat. No. 10006788), the Ligation sequencing kit (SQK-LSK109) and Native Barcoding Expansion 1-12 kit (EXP-NBD104) from Oxford Nanopore Technologies and ordered the 3. part reagents from New England Biolabs; Q5 Hot Start High-Fidelity 2X Master Mix (M0494L), LunaScript RT SuperMix Kit (E3010L), NEBNext® Ultra™ II End Repair/dA-Tailing Module (E7546L), NEBNext® Quick Ligation Module (E6056L) and Blunt/TA Ligase Master Mix (M0367L). The samples are loaded on a spot on Mk 1 R9 Version Flow Cell (Cat. No. FLO- MIN106D) and sequenced on a GridION device. For bioinformatic analysis, the fast5 files were basecalled and demultiplexed using guppy 4.3.4+ecb2805 on the GridION, with the flag to require barcodes on both ends turned on. We then used an in-house pipeline (33) which runs artic v1.2.1 (34) and then uses a QC script (35) to count number of aligned reads, base coverage and percentage of Ns. Any genomes with less than 90% of bases called with >20X reads are

then excluded, and lineage assignment is performed with pangolin (latest release) (16) and clade assignment with Nextclade CLI (latest release) (36).To define the clusters we compared the SNPs and deletions between the sequences belonging to the same lineages as reported by Nextclade. Sequences were deemed to belong to one cluster if they had maximally 0-1 SNP difference.

Centre 12

Nucleic acid extraction was performed using the Chemagic360™ platform and chemagic™ Viral DNA/RNA 300 Kit H96 extraction kit (PerkinElmer/Wallac, Turku, Finland). NGS library preparation was performed with QIAseq SARS-CoV-2 Primer Panel (QIAGEN, USA), the quality of the library was determined with QIAxcel DNA High Resolution Kit (QIAGEN) and Qubit™ dsDNA HS Assay Kit (Invitrogen™). Sequencing was performed with Illumina™ Miniseq platform using Miniseq Mid Output kit (300 cycles) (Illumina™, USA). Results were analyzed with Illumina BaseSpace application DRAGEN COVID Lineage and comparison was done with Nextclade software. The cluster assignment was based on the Nextclade and the DRAGEN COVID Lineage output.

Centre 13

Nucleic acid were extracted using the MagMAX Viral/Pathogen kit (Applied biosystems) from 200 ul of initial sample on a KingFisher Presto instrument (Thermo Fisher Scientific) integrated in the Nimbus Presto workstation (Hamilton). Nucleic acids were eluted in 50 ul and stored at -20°C before sequencing analysis. Then, 8.5 ul of eluates were used to prepare

the libraries using the Illumina COVIDSeq Test library preparation reagents (Illumina) according to the manufacturer's instructions. Libraries were sequenced on the Illumina NovaSeq 6000 SP flow cell, normally pooling 384 libraries per lane, using a 2x59-nt sequencing protocol. Paired reads were quality filtered and then analysed using an in-house processing pipeline developped by the Health 2030 Genome Center in Geneva (37). Identification of clusters: complete genomes were automatically translated into proteins. Spike proteins were aligned using MAFFT and a phylogenetic Neighbour Joining tree was calculated. The clusters in the tree were identified by comparing signature substitutions/deletions in the alignment.

Centre 14

RNA was extracted using the MagDEA Dx SV kit on Maglead platform (PSS bio system net) according to manufacturer's instructions. A volume of 280ul lysis buffer was added to 220ul sample, and eluted in 50 µL. Sequencing libraries were prepared using the Illumina COVIDSeq Test, and sequenced on Novaseq 6000 producing at least 3.3 million paired end reads (150nt) per library.

Library quality was analyzed using FastQC (version 0.11.8, Babraham Bioinformatics). Reads were aligned to the genome using Bowtie2 (version 2.3.4.3) with the command options: -k 4 --no-discordant. reads with more than 6 variants in 100 bases were discarded (SNV, deletion or insertion each count as one variant). Variants were called using ivar variants (version 1.3.1). Consensus sequence was built based on the ivar variants table using the R Biostrings package according to these rules: Positions with less than 10 reads were called as N.

Variants with frequency higher than 0.7 were included in the consensus sequence. Variants with frequency between 0.3 and 0.7 and at least 50 reads were considered as "wobbles" using the IUPAC letters. Consensus sequences were submitted to Pangolin command-line tool (pangolin version 2.2.2 and pangoLEARN version 2021-02-12) and Nextclade (version 0.12.0) to determine the PANGO lineage and clade. Consensus sequences were aligned and a phylogenetic tree was built using ngphylogeny.fr – PhyML+SMS workflow, which is based on a maximum likelihood reference. Cluster identification was determined by samples having a shared ancestor on phylogenetic tree.

Centre 15

Nucleic acids were extracted using the MagNA Pure 96 system and the DNA and viral RNA small volume kit (Roche Diagnostics, Rotkreuz, Switzerland) or using the Abbott m2000 Realtime System and the Abbott sample preparation system reagent kit (Abbott, Baar, Switzerland). Amplicon sequencing followed the ARTIC nCOV-2019 protocol with a weighted v3 primer mix. Libraries were prepared with the Illumina DNA Prep kit (Illumina) on a Hamilton STAR robot. Up to 96 samples were pooled equimolarly and sequenced paired-end 150bp on an Illumina NextSeq 500 mid output flow cell.

Reads were demultiplexed with bcl2fastq v.2.17 (Illumina) and assembled using the COVGAP Pipeline (v10.6) (38) as previously described in (39,40). Briefly, a minimal depth of 50 was required for bases to be called. SNPs were called with a minimum allele frequency of 0.7. while ambiguous bases with lower allele frequency were masked for further analysis.

288 Clusters were identified by calculating a maximum likelihood tree using RAxML with a

289 maximum difference of 1 SNP between sequences.

References

1. Quick J. nCoV-2019 sequencing protocol v3 (LoCost) V.3. https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye. 2020.
2. SmaltAlign. https://github.com/medvir/SmaltAlign.
3. Loman N, Rambaut A. nCoV-2019 novel coronavirus bioinformatics protocol. https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html.
4. Grädel C, Miani MAT, Barbani MT, Leib SL, Suter-Riniker F, Ramette A. Rapid and cost-efficient enterovirus genotyping from clinical samples using flongle flow cells. Genes. 2019;10(9).
5. Neuenschwander SM, Miani MAT, Amlang H, Perroulaz C, Bittel P, Casanova C, et al. A sample-to-report solution for taxonomic identification of cultured bacteria in the clinical setting based on nanopore sequencing. Journal of Clinical Microbiology. 2020;58(6).
6. Medaka. https://github.com/nanoporetech/medaka.
7. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics. 2011;27(21).
8. GENCOV. https://github.com/metagenlab/GENCOV.
9. CoVpipe. https://gitlab.com/RKIBioinformaticsPipelines/ncov_minipipe.
10. Chen S, Zhou Y, Chen Y, Gu J. Fastp: An ultra-fast all-in-one FASTQ preprocessor. In: Bioinformatics. 2018.
11. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint arXiv. 2013;
12. Okonechnikov K, Conesa A, García-Alcalde F. Qualimap 2: Advanced multi-sample quality control for high-throughput sequencing data. Bioinformatics. 2016;32(2).
13. fgbio. https://github.com/fulcrumgenomics/fgbio.
14. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing -- Free bayes -- Variant Calling -- Longranger. arXiv preprint arXiv:12073907. 2012;
15. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. GigaScience. 2021;10(2).
16. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nature Microbiology. 2020;5(11).
17. ARTIC nanopore protocol for nCoV2019 novel coronavirus. https://github.com/artic-network/artic-ncov2019.
18. Jalili V, Afgan E, Gu Q, Clements D, Blankenberg D, Goecks J, et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update. Nucleic Acids Research. 2021;48(W1).
19. Maier W, Bray S, van den Beek M, Bouvier D, Coraor N, Miladi M, et al. Freely accessible ready to use global infrastructure for SARS-CoV-2 monitoring. bioRxiv. 2021;
20. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14).
21. iVar. https://andersen-lab.github.io/ivar/html/manualpage.html.

22. Wilm A, Aw PPK, Bertrand D, Yeo GHT, Ong SH, Wong CH, et al. LoFreq: A sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Research. 2012;40(22).
23. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly. 2012;6(2).
24. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25(16).
25. GitHub: SARS-CoV-2 Analyses. https://github.com/jonas-fuchs/SARS-CoV-2-analyses.
26. Oude Munnink BB, Nieuwenhuijse DF, Stein M, O'Toole Á, Haverkate M, Mollers M, et al. Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands. Nature Medicine. 2020;26(9).
27. MACOVID. https://github.com/MUMC-MEDMIC/MACOVID.
28. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25(3).
29. Coolen JPM, Wolters F, Tostmann A, van Groningen LFJ, Bleeker-Rovers CP, Tan ECTH, et al. SARS-CoV-2 whole-genome sequencing using reverse complement PCR: For easy, fast and accurate outbreak and variant analysis. Journal of Clinical Virology. 2021;144.
30. Easyseq. https://github.com/JordyCoolen/easyseq_covid19.
31. Freed NE, Vlková M, Faisal MB, Silander OK. Rapid and inexpensive whole-genome sequencing of SARS-CoV-2 using 1200 bp tiled amplicons and Oxford Nanopore Rapid Barcoding. Biology Methods and Protocols. 2021;5(1).
32. Pedersen BS, Quinlan AR. Mosdepth: Quick coverage calculation for genomes and exomes. Bioinformatics. 2018;34(5).
33. SusCovONT. https://github.com/marithetland/susCovONT.
34. The ARTIC field bioinformatics pipeline. https://github.com/artic-network/fieldbioinformatics.
35. Connor lab: QC script. https://github.com/connor-lab/ncov2019-artic-nf/blob/master/bin/qc.py.
36. Aksamentov I, Neher R. Nextclade. https://github.com/nextstrain/nextclade. 2020.
37. Health 2030 Genome Center SARS-CoV2 pipeline. https://github.com/health2030genomecenter/SARS-CoV-2_pipeline.
38. Mari A. COVGAP. https://github.com/appliedmicrobiologyresearch/covgap.
39. Stange M, Marii A, Roloff T, Seth-Smith HMB, Schweitzer M, Brunner M, et al. SARS-CoV-2 outbreak in a tri-national urban area is dominated by a B.1 lineage variant linked to a mass gathering event. PLoS Pathogens. 2021;17(3).
40. Mari A, Roloff T, Stange M, Søgaard KK, Asllanaj E, Tauriello G, et al. Global Genomic Analysis of SARS-CoV-2 RNA Dependent RNA Polymerase Evolution and Antiviral Drug Resistance. Microorganisms. 2021 May 19;9(5).

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Supplemental Tables

Sample	Ct
NGS1	22
NGS2	21.5
NGS3	20.8
NGS4	19.4
NGS5	19.9
NGS6	21.1
NGS7	27.1
NGS8	28
NGS9	28.4
NGS10	28.1

Suppl. table S1: Samples and viral load as measured by qPCR provided to the participating laboratories.

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Centers	NGS1	NGS2	NGS3	NGS4	NGS5	NGS6	NGS7	NGS8	NGS9	NGS10	Mean	SD
1	3080	3628	3051	3546	3200	4183	3780	3268	4468	4347	3655	527
2	406	412	409	413	417	408	393	349	391	383	398	21
3	7558	7493	7915	7099	7132	8061	7462	7755	7985	7907	7637	344
4	3011	3131	2898	3659	3669	3320	2618	1583	2069	862	2682	918
5	2999	2766	3678	3696	2597	5062	3600	2556	3346	2124	3242	835
6	2996	1752	2854	2930	2112	2440	1917	2996	3113	2819	2593	500
7	862	191	139	171	131	240	525	39	703	246	325	275
8	896	807	1414	982	1222	1047	1346	260	579	424	898	386
9	392	396	301	400	412	375	361	9	334	148	313	132
10	1196	1209	1210	1223	1210	1215	1186	796	1077	1082	1140	132
11	960	963	690	932	908	1028	1038	448	888	1017	887	184
12	3523	2990	2893	2759	2890	3014	2556	3078	3366	3510	3058	320
13	6044	5012	5093	6052	6316	6079	6119	5530	5844	6144	5823	457
14	39294	36491	29619	41782	37390	36710	42894	36949	34715	35877	37172	3712
15	2355	2256	2529	2680	2864	2685	1175	1322	1660	1364	2089	644

Suppl. table S2: Mean read depth for each sample and centre.

Centre	NGS1	NGS2	NGS3	NGS4	NGS5	NGS6	NGS7	NGS8	NGS9	NGS10
1	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.28	0.27	0.52
2	0.00	0.00	0.84	1.58	0.00	0.84	0.00	2.61	1.79	1.74
3	0.02	0.02	0.02	0.01	0.03	0.02	0.04	0.12	0.05	0.07
4	0.26	0.22	0.22	0.22	0.22	0.22	0.22	0.45	0.29	1.12
5	0.11	0.11	0.41	0.10	0.10	0.10	0.10	1.30	0.12	0.69
6	0.80	0.65	3.02	0.00	0.00	0.00	0.65	0.64	1.39	0.65
7	2.63	1.64	9.31	1.19	1.44	1.06	6.21	43.18	3.39	7.95
8	0.62	0.62	0.62	0.62	0.62	0.62	0.65	1.15	0.74	1.19
9	0.63	0.63	3.26	3.40	0.63	3.26	3.98	99.91	7.49	0.64
10	0.40	0.41	0.40	0.40	0.40	0.40	0.40	2.24	0.40	1.34
11	1.40	0.41	2.24	1.99	0.40	1.26	0.42	5.80	3.35	1.34
12	0.27	0.13	0.14	0.14	0.13	0.13	0.68	1.37	0.13	0.75
13	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.69	0.25	1.03
14	0.26	0.27	0.27	0.24	0.26	0.56	0.26	0.96	0.27	1.08
15	0.30	0.30	1.04	0.31	0.30	1.04	0.30	4.61	1.26	1.30

Suppl. table S3: Percentage of missing data (Ns) in consensus genomes.

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Centre	NGS1	NGS2	NGS3	NGS4	NGS5	NGS6	NGS7	NGS8	NGS9	NGS10	mean
1	0.83	0.98	1.00	0.92	1.00	1.00	0.78	0.83	0.81	0.62	0.88
2	0.93	0.95	0.94	0.92	1.00	0.94	0.78	0.71	0.77	0.54	0.85
3	0.87	0.95	0.94	0.92	1.00	0.94	0.78	0.86	0.81	0.63	0.87
4	0.93	1.00	1.00	1.00	1.00	1.00	0.97	0.86	1.00	0.95	0.97
5	0.93	1.00	0.89	1.00	1.00	0.94	0.97	0.71	1.00	0.88	0.93
6	0.93	0.95	1.00	0.92	1.00	1.00	0.81	0.86	0.84	0.63	0.89
7	0.87	1.00	0.56	0.92	1.00	0.83	0.69	0.24	0.81	0.63	0.75
8	0.93	1.00	0.89	0.88	1.00	0.89	0.69	0.76	0.71	0.63	0.84
9	0.93	1.00	0.94	0.92	1.00	0.94	0.75	0.00	0.74	0.63	0.79
10	0.93	0.95	1.00	0.92	1.00	1.00	0.78	0.81	0.81	0.54	0.87
11	0.87	1.00	0.94	0.92	1.00	0.94	0.63	0.81	0.81	0.61	0.85
12	0.93	1.00	1.00	0.92	1.00	1.00	0.75	0.76	0.81	0.56	0.87
13	0.93	1.00	1.00	1.00	1.00	1.00	0.97	0.81	1.00	0.90	0.96
14	0.87	1.00	1.00	1.00	1.00	0.94	0.97	0.71	1.00	0.90	0.94
15	0.93	1.00	0.94	1.00	1.00	0.94	0.97	0.57	1.00	0.78	0.91
mean	0.91	0.99	0.94	0.94	1.00	0.96	0.81	0.69	0.85	0.68	

Suppl. table S4: Variant calling score for each sample and centre and mean score per centre.

centre	NGS1	NGS2	NGS3	NGS4	NGS5	NGS6	NGS7	NGS8	NGS9	NGS10
1	13 | 2 | 0	20 | 1 | 0	18 | 0 | 0	23 | 1 | 0	20 | 0 | 0	18 | 0 | 0	28 | 3 | 0	20 | 1 | 0	28 | 3 | 0	31 | 7 | 1
2	15 | 0 | 0	20 | 0 | 1	17 | 0 | 1	23 | 1 | 0	20 | 0 | 0	17 | 0 | 1	28 | 3 | 0	18 | 0 | 3	27 | 3 | 1	29 | 7 | 3
3	13 | 0 | 2	20 | 0 | 1	17 | 0 | 1	23 | 1 | 0	20 | 0 | 0	17 | 0 | 1	28 | 3 | 0	21 | 0 | 0	28 | 3 | 0	32 | 6 | 1
4	15 | 0 | 0	21 | 0 | 0	18 | 0 | 0	24 | 0 | 0	20 | 0 | 0	18 | 0 | 0	31 | 0 | 0	21 | 0 | 0	31 | 0 | 0	39 | 0 | 0
5	14 | 0 | 1	21 | 0 | 0	16 | 0 | 2	24 | 0 | 0	20 | 0 | 0	17 | 0 | 1	31 | 0 | 0	19 | 1 | 1	31 | 0 | 0	36 | 0 | 3
6	15 | 0 | 0	20 | 0 | 1	18 | 0 | 0	23 | 1 | 0	20 | 0 | 0	18 | 0 | 0	28 | 2 | 1	21 | 0 | 0	28 | 2 | 1	32 | 6 | 1
7	14 | 0 | 1	21 | 0 | 0	11 | 1 | 6	22 | 0 | 2	20 | 0 | 0	16 | 1 | 1	25 | 3 | 3	8 | 0 | 13	27 | 2 | 2	31 | 5 | 3
8	15 | 0 | 0	21 | 0 | 0	17 | 1 | 0	22 | 1 | 1	20 | 0 | 0	17 | 1 | 0	26 | 4 | 1	20 | 1 | 0	26 | 4 | 1	32 | 6 | 1
9	15 | 0 | 0	21 | 0 | 0	17 | 0 | 1	23 | 1 | 0	20 | 0 | 0	17 | 0 | 1	27 | 3 | 1	0 | 0 | 21	26 | 3 | 2	32 | 6 | 1
10	15 | 0 | 0	20 | 0 | 1	18 | 0 | 0	23 | 1 | 0	20 | 0 | 0	18 | 0 | 0	28 | 3 | 0	20 | 0 | 1	28 | 3 | 0	29 | 7 | 3
11	14 | 0 | 1	21 | 0 | 0	17 | 0 | 1	23 | 1 | 0	20 | 0 | 0	17 | 0 | 1	23 | 3 | 5	18 | 0 | 3	28 | 3 | 0	31 | 6 | 2
12	15 | 0 | 0	21 | 0 | 0	18 | 0 | 0	23 | 1 | 0	20 | 0 | 0	18 | 0 | 0	27 | 3 | 1	20 | 1 | 0	28 | 3 | 0	30 | 7 | 2
13	15 | 0 | 0	21 | 0 | 0	18 | 0 | 0	24 | 0 | 0	20 | 0 | 0	18 | 0 | 0	31 | 0 | 0	20 | 0 | 1	31 | 0 | 0	36 | 0 | 2
14	14 | 1 | 0	21 | 0 | 0	18 | 0 | 0	24 | 0 | 0	20 | 0 | 0	17 | 0 | 1	31 | 0 | 0	18 | 2 | 1	31 | 0 | 0	39 | 0 | 0
15	15 | 0 | 0	21 | 0 | 0	17 | 0 | 1	24 | 0 | 0	20 | 0 | 0	17 | 0 | 1	31 | 0 | 0	17 | 3 | 1	31 | 0 | 0	33 | 1 | 5

Suppl. Table S5: Count of (correct | wrong | missing) SNP calls for each sample and centre.