

1 External quality assessment of SARS-CoV-2-sequencing: An ESGMD-

2 SSM pilot trial across 15 European laboratories

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80 Abstract

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

85

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

90

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

98

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

104 Introduction

105

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

113

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

124

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

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

132

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

- 136 (i) identification of SNPs and deletions,
- 137 (ii) identification of Pango lineages (8), and
- 138 (iii) assessing the genomic relatedness using a molecular epidemiological
139 approach.

140

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

144 **Methods and Materials**

145

146 **Design of the external quality assessment**

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

151

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

154

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

159

160 **Samples**

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

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

169

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

178

179 *Assessment of variant calling*

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

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

192

193 Assessment of lineage and cluster assignment

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

199 Results

200

201 Genome depth, coverage and assembly

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

207

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

212

213 SNPs and Indels

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

221

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

235 Lineage assignment

236 Correct lineage assessment is of course dependent on correct SNP calling and sufficient
237 coverage across the genome. The majority of centres assigned all samples to the correct
238 lineage (**table 2**). Two centres with the lowest mean depth failed in correctly assigning the
239 lineage of one sample, NGS8 (B.1.177; **Supplementary Table S2**). Centre 7, which provided a
240 57% complete genome (mean read depth 39x), could assign the sample to lineage B. Rather
241 surprisingly, the laboratory with the by far highest depth, centre 14, assigned the lineages of
242 two samples incorrectly: NGS7 and -9 were both only assigned as lineages A, as opposed to
243 the more accurate “correct solution” of A.27. This was due to an outdated version of
244 pangolin.

245

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.

262 Discussion

263

264 Impact of methodological choices

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

271

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

280

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

286 genotyping. For example, samples NGS2 and -5 for centre 7 have 191x and 131x,
287 respectively, as well as a low amount of missing data, and a high variant calling score (**figure**
288 **1**). However, when coverage is uneven, missing data can still be an issue even at higher
289 average depth (e.g., NGS10 for centre7 at 246x, **figure 1, Supplementary table S2**). For
290 accurately genotyping SARS-CoV-2, it is necessary to capture the entirety of the genome and
291 not just some areas (even of biologically important such as the S gene) as the software used
292 to determine the lineage built its models based on whole genome diversity (the
293 pangoleARN algorithm within pangolin) (8). It is therefore important to strive for the best
294 coverage across the genome (i.e., a low amount of missing data) and “sufficient read
295 depth”, as mentioned above, is therefore a function of this. More even coverage in
296 amplicon-based sequencing can for example be achieved by balancing primer sets.
297
298 Instead of average depth, other factors such as variant reporting capacity, mapping quality
299 as well as interpretation of data play a larger role. This is an important point for diagnostic
300 labs with respect to operational costs. The importance of this was highlighted by centre 14
301 which sequenced to the by far highest depth but had nevertheless difficulties with lineage
302 and cluster assignment despite very good variant calling. Upon receiving a preliminary
303 report, centre 14 re-examined their analysis pipeline and found they had used an outdated
304 Pangolin and pangoleARN version. The Pango lineage nomenclature is dynamic, meaning
305 that nomenclature system develops as SARS-CoV-2 evolves, and lineage definitions and
306 names can change over time (8). The pilot EQA provided here valuable feedback for the
307 respective centre to improve its workflows.
308

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 and 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

333 All protocols used by the participating centres in this EQA used amplicon-based sequencing,
334 and primer bias can have an influence on sequencing accuracy. Here, primer sets vary
335 between labs (**table 1**). For the ARTIC v3 primers (which are public), we find no apparent bias
336 in the data reported here compared to the other primer panels. However, centres 7 and 8
337 which used the same primer panel but did not detect the variant G21255C in samples NGS3,
338 -6 and -8 (**Supplementary figure 1C, F, H**). This SNP is present in almost all representatives
339 of lineage B.1.177 (12). Whether this failure in detection is truly due to a primer bias cannot
340 be conclusively answered though, as commercial primer sequences are often not public. A
341 possibility to deal with this issue bioinformatically is to trim primer sequences prior to
342 assembly. Nevertheless, primer bias is a real issue if it leads to dropouts. Fortunately, it is
343 actively monitored by the community. For example, dropouts of the ARTIC v3 panel have
344 been reported especially for Beta and Delta variants. For this reason, a new primer panel
345 has been developed to avoid high frequency variant sites in the newer lineages (13).

346

347 Factors not assessed in this pilot EQA

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

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

362

363 Conclusion and lessons learnt

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

373

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

381

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

390

391

392

393 [Conflict and interest statement & acknowledgements.](#)

394

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487

488 [Figure Captions](#)

489

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

497

498 [Tables](#)

499

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)

500 Table 1: Summary of the methods used by the participating centres. A detailed method

501 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	B.1.36.17	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

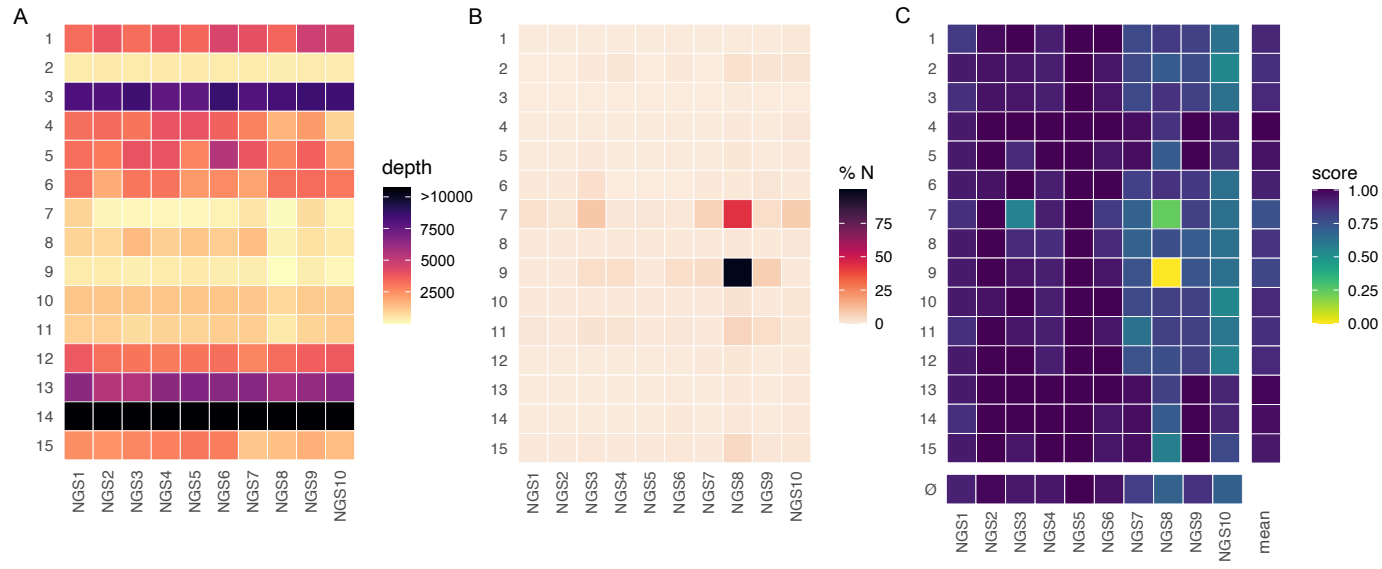
Centre	NGS1	NGS2	NGS3	NGS4	NGS5	NGS6	NGS7	NGS8	NGS9	NGS10
1	A	B	C	D	B	C	E	C	E	F
2	A	B	C	D	B	C	E	C	E	F
3	A	B	C	D	B	C	E	C	E	F
4	A	B	C	D	B	C	E	C	E	F
5	A	B	C	D	B	C	E	C	E	F
6	A	B	C	D	B	C	E	C	E	F
7	A	B	C	D	B	C	E	C*	E	F
8	A	B	C	D	B	C	E	C	E	F
9	A	B	C	D	B	C	E	N/A	E	F
10	A	B	C	D	B	C	E	C	E	F
11	A	B	C	D	B	C	E	C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

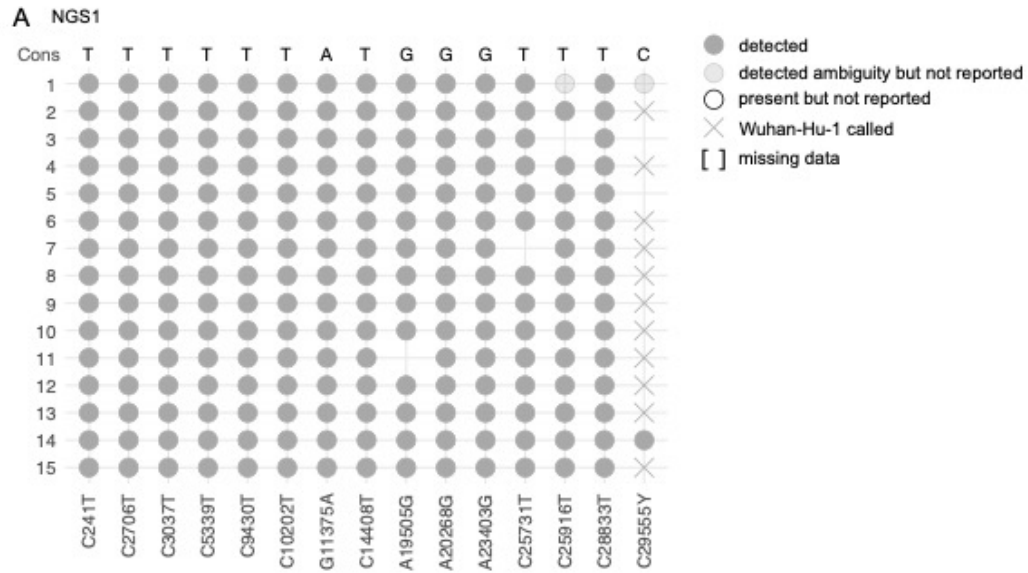
507 main text. The * marks that the centre reported an assumed cluster assignment based on a

508 partial genome.

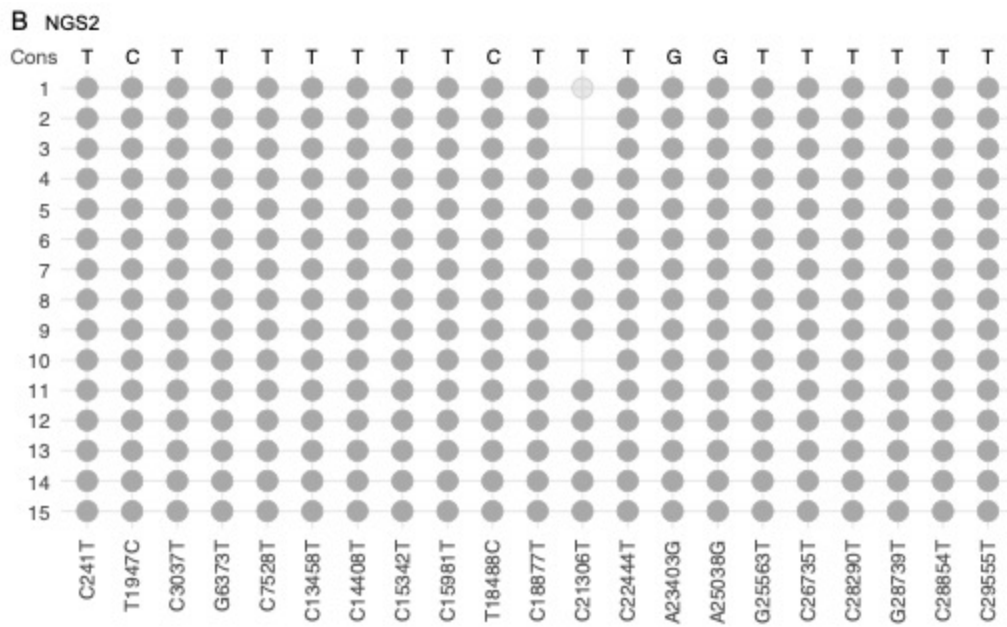


1 Supplemental Figures

2

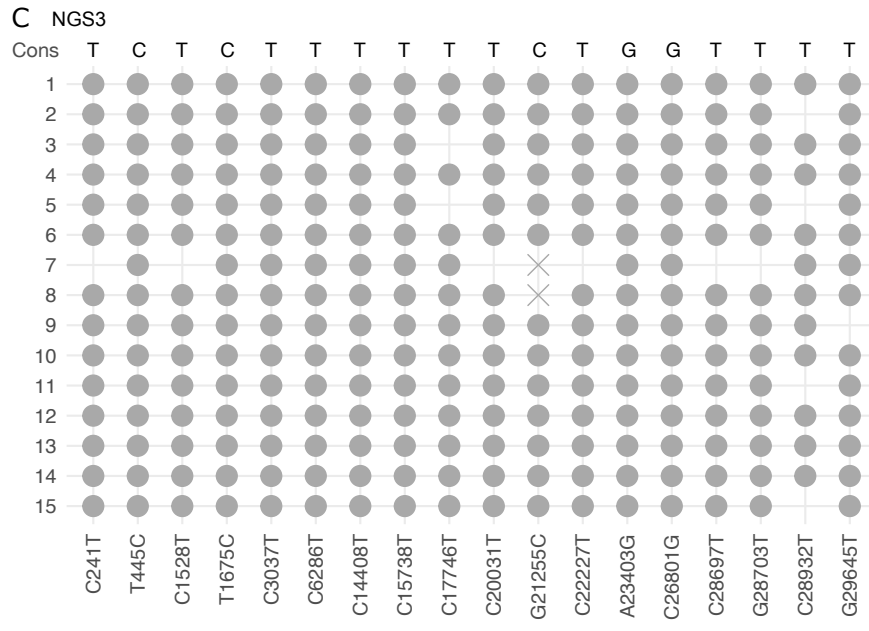


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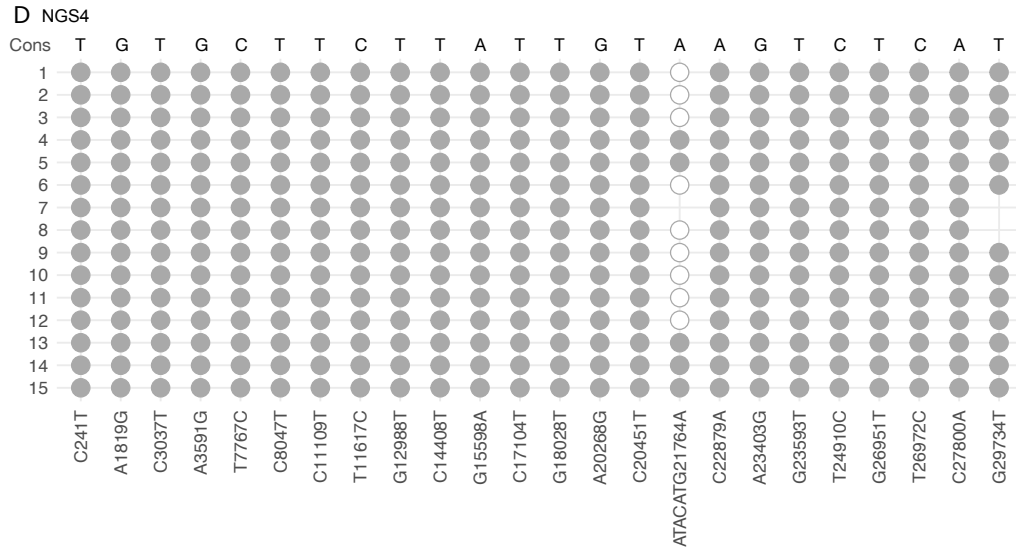


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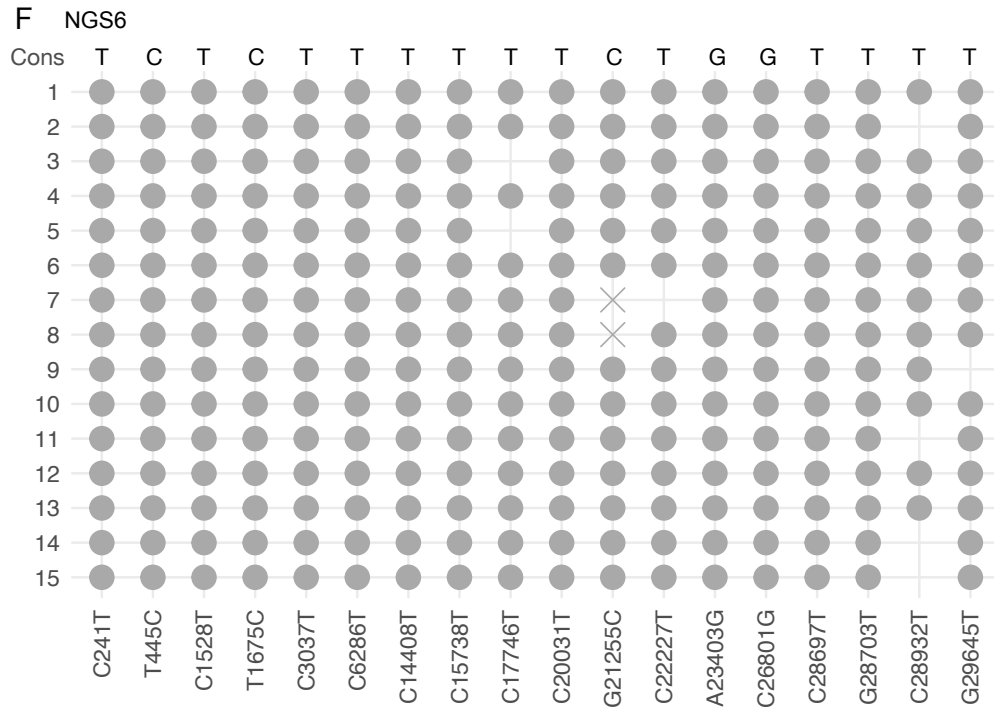
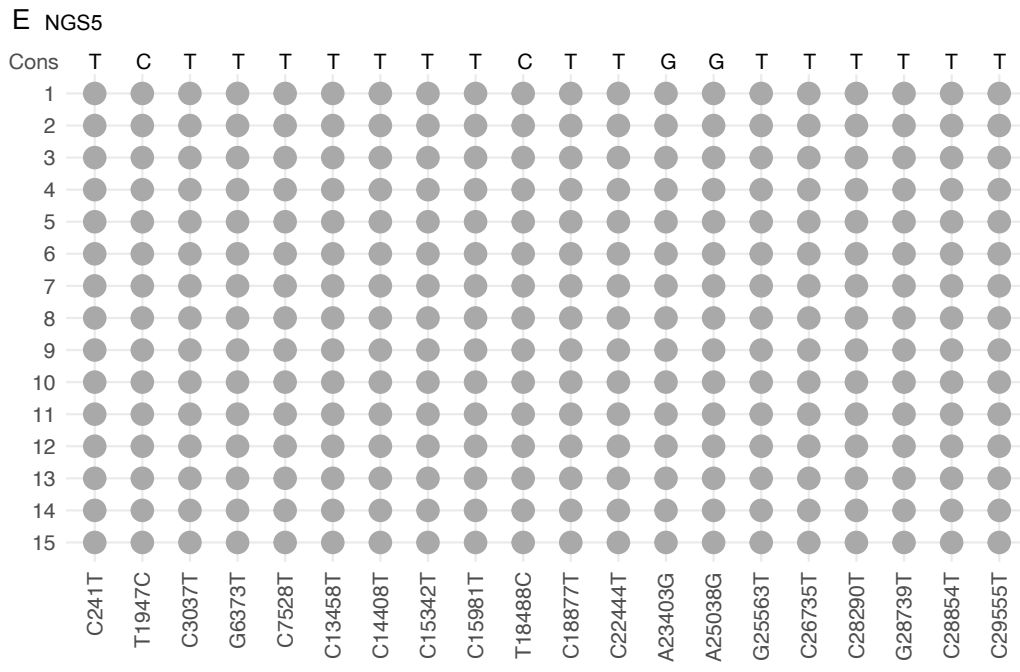


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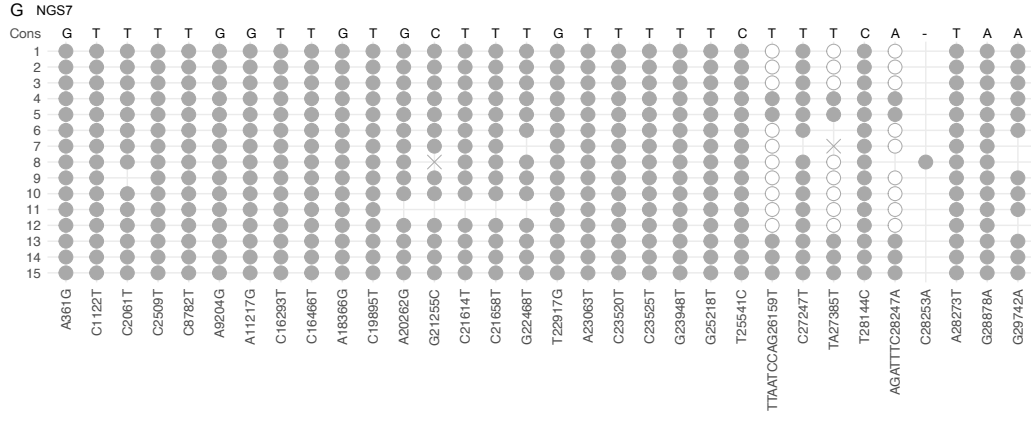


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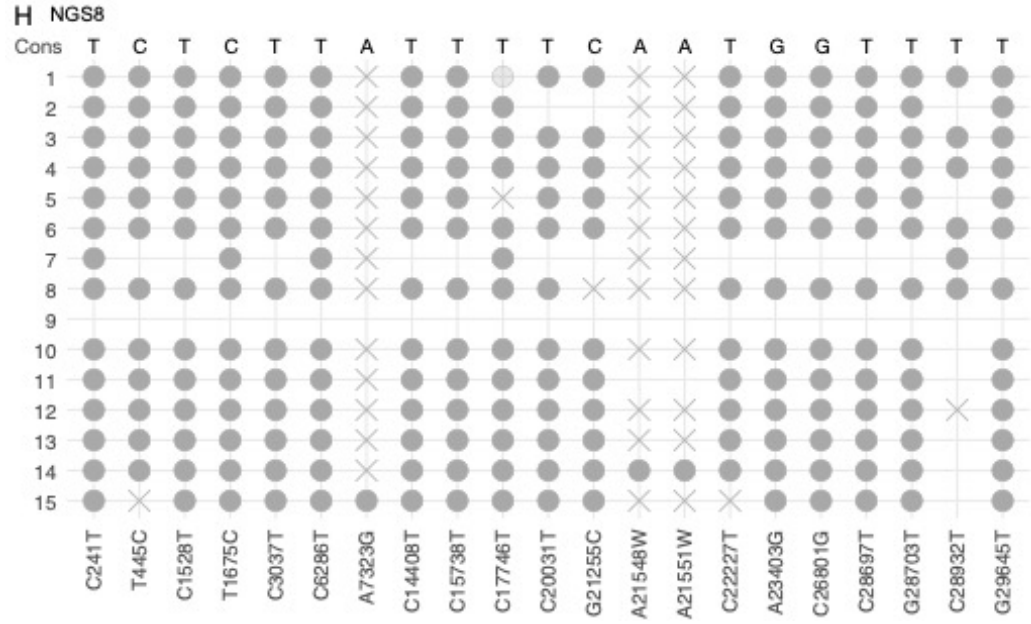
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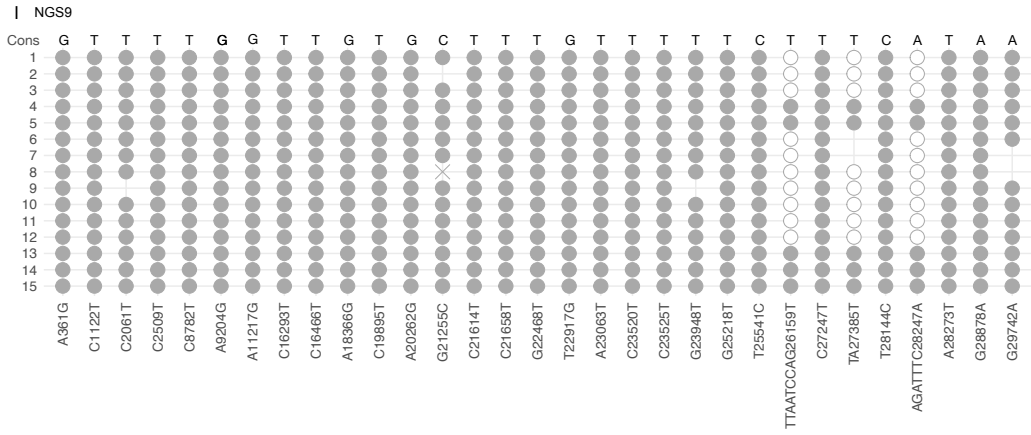
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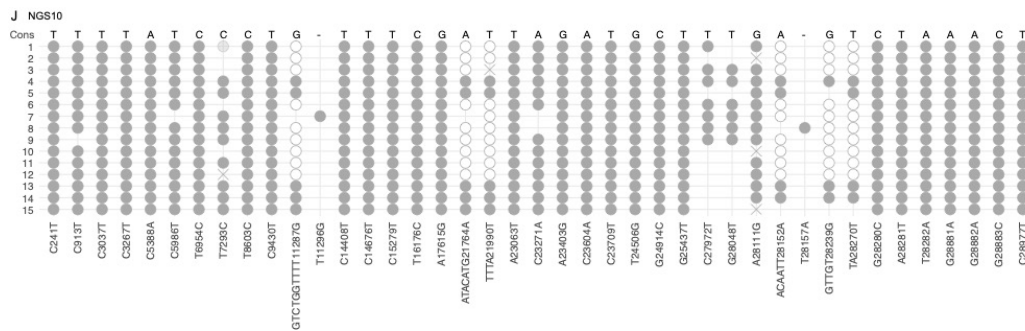


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12

13 Suppl. figure S1: A-J Presence and absence of SNPs and Indels per sample. On the x-axis, all

14 variations that were specifically reported by the centres are listed. On the y-axis are the

15 centres. A dark grey filled circle means the respective SNP was reported. No symbol means

16 the genome sequence has an N at that position. A cross indicates that instead of the SNP,

17 the reference position was called; this can either be because the SNP is not true or because

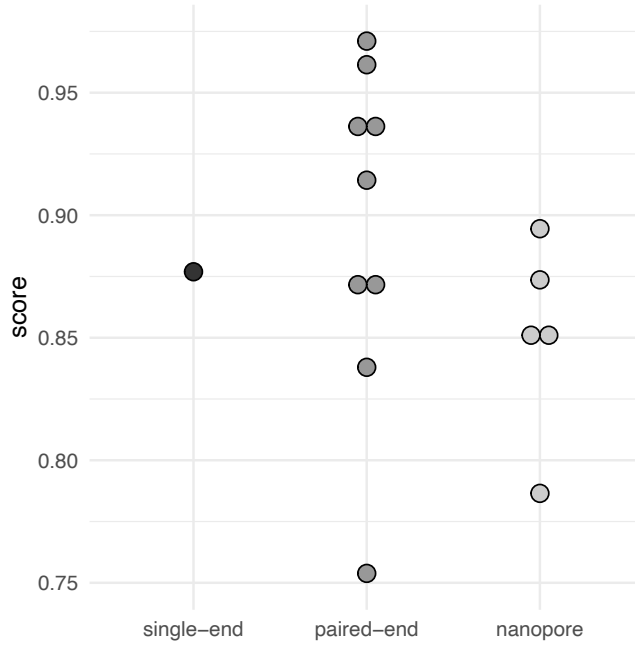
18 the base call is wrong. Additionally, sometimes ambiguous sites were reported as SNPs or

19 are present in the consensus genome at the position of a reported SNP. If such a position

20 was found in the sequence (but not reported) a less opaque filled circle is shown. Lastly,

21 some centres did not report deletions. If these non-reported deletions were nevertheless

22 present in the data, they are indicated with a white-filled circle.

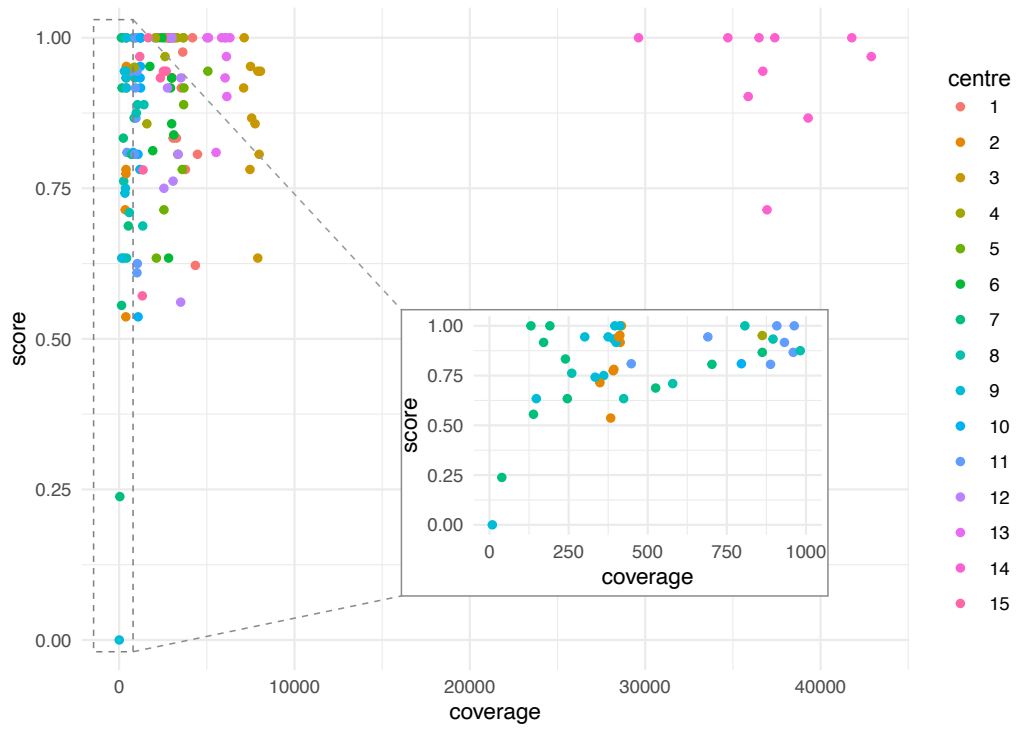


23

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

25 used.

26



27

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

29 coverage.

1 Supplemental Methods

2

3

4 Centre 1

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

14

15

16 Centre 2

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

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

32

33

34 Centre 3

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

44

45

46 Centre 4

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

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

63

64

65 Centre 5

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

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

75

76 **Bioinformatics.** The de-multiplexed raw reads were subjected to a custom Galaxy pipeline
77 (18,19). The raw reads were pre-processed with fastp (v.0.20.1) (10) and mapped to the
78 SARS-CoV-2 Wuhan-Hu-1 reference genome (Genbank: NC_045512) using BWA-MEM
79 (v.0.7.17) (20). For datasets, which were produced with the ARTIC v3 protocol, primer
80 sequences were trimmed with ivar trim (v1.9) (21). Variants (SNPs and INDELS) were called
81 with the ultrasensitive variant caller LoFreq (v2.1.5) (22) demanding a minimum base quality
82 of 30 and a coverage of at least 5-fold. Afterwards, the called variants were filtered based
83 on a minimum variant frequency of 10 % and on the support of strand bias. The effects of
84 the mutations were automatically annotated in the vcf files with SnpEff (v.4.3.1) (23).
85 Finally, consensus sequences were constructed by bcftools (v.1.1.0) (24). Regions with low
86 coverage >5x or variant frequencies between 30 and 70 % were masked with Ns. The variant
87 frequencies (>10%) of the nucleotide substitutions of the respective samples were matched
88 in a matrix and clusters were determined by hierarchical clusterin (ward.D2) using the R
89 package hclust. The script is available on GitHub (25)and was implemented on usegalaxy.eu.

90

91

92 Centre 6

93 Sequencing of SARS-CoV-2-positive samples

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

97 Kit 96 (Roche, Germany) on the MagNA Pure 96 system (Roche, Germany), without the
98 addition of an internal extraction control.
99
100 Sequencing was performed using the PCR tiling of SARS-CoV-2 virus with Native Barcoding
101 Expansion 96 (EXP-NBD196) protocol (Version: PTCN_9103_v109_revH_13Jul2020) of
102 Oxford Nanopore technologies, with minor modifications and using the primers previously
103 published by Oude Munnink et al. (26). Briefly, the only modifications were extending the
104 barcode and adaptor ligation steps up to 60 min and loading 48 samples per flow cell.
105
106 Bioinformatic analysis was performed using an in-house developed pipeline MACOVID that
107 is based on Artic v1.1.3. In brief, short and obvious chimeric reads are filtered with Cutadapt
108 v2.5. The filtered reads were mapped to the reference genome MN908947.3 with Minimap2
109 v2.17 and quality checked with “align_trim” function of Artic v1.1.3. Mapped reads were
110 split per primer pool using Samtools v1.9 and a consensus was created per primer pool with
111 Medaka v1.0.3. Variants were called using Medaka v1.0.3 and Longshot v0.4.1. Low
112 coverage regions (<30x) were masked with “artic_make_depth_mask” function of Artic
113 v1.1.3. A preconsensus was made with “artic_mask” and the final consensus sequence was
114 made with bcftools v1.10.2. Documentation and source code are available from (27) under
115 MIT license. The consensus sequences were used to construct a phylogenetic tree with the
116 ncov pipeline v3 of nextstrain. Samples were considered to be part of the same cluster of
117 there are ≤ 2 SNPs difference. Pangolin lineages were assigned were assigned using the
118 Pangolin COVID-19 Lineage Assigner web application on <https://pangolin.cog-uk.io/>.
119
120

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; SLIDINGWINDOW: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.

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

149

150 Centre 8

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

161

162

163 Centre 9

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

168

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

174

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

179

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

182

183

184 Centre 10

185 RNA was isolated using an easyMAG extractor following manufacturer's instructions for
186 extraction of total nucleic acids from airways samples (BioMérieux, Marcy-l'Étoile, France).

187 Detection of SARS-CoV-2 virus was performed using a validated qualitative RT-PCR detecting
188 the SARS-CoV-2 virus E-gene based on a method published by Corman et al (28). Eluted RNA
189 was reverse transcribed and PCR amplified according to the ARTIC Network v3 protocol using
190 the ARTIC nCoV-2019 version 3 primer set with annealing temperature at 63 °C during PCR.

191 The PCR products were sequenced on a GridION sequencer (Oxford Nanopore Technologies,
192 Oxford, UK). The Medaka-pipeline by the ARTIC network (3) was used to generate consensus

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

199

200

201 Centre 11

202 RNA were extracted on a Biomek i7 automated workstation (Beckman Coulter) using
203 their RNAdvanceViral kit (C63510) and protocol (and a Ct value from an in house Sarbeco-
204 PCR provided). Further, we performed the ARTIC protocol v3 for PCR and library prep (1)
205 using the ARTIC nCoV-2019 v3 primer panel from Integrated DNA technologies (Cat. No.
206 10006788), the Ligation sequencing kit (SQK-LSK109) and Native Barcoding Expansion 1-12
207 kit (EXP-NBD104) from Oxford Nanopore Technologies and ordered the 3. part reagents
208 from New England Biolabs; Q5 Hot Start High-Fidelity 2X Master Mix (M0494L), LunaScript
209 RT SuperMix Kit (E3010L), NEBNext® Ultra™ II End Repair/dA-Tailing Module (E7546L),
210 NEBNext® Quick Ligation Module (E6056L) and Blunt/TA Ligase Master Mix (M0367L). The
211 samples are loaded on a spot on Mk 1 R9 Version Flow Cell (Cat. No. FLO- MIN106D) and
212 sequenced on a GridION device. For bioinformatic analysis, the fast5 files were basecalled
213 and demultiplexed using guppy 4.3.4+ecb2805 on the GridION, with the flag to require
214 barcodes on both ends turned on. We then used an in-house pipeline (33) which runs artic
215 v1.2.1 (34) and then uses a QC script (35) to count number of aligned reads, base coverage
216 and percentage of Ns. Any genomes with less than 90% of bases called with >20X reads are

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

222

223

224 Centre 12

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

234

235

236 Centre 13

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

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

250

251

252 Centre 14

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

258

259 Library quality was analyzed using FastQC (version 0.11.8, Babraham Bioinformatics). Reads
260 were aligned to the genome using Bowtie2 (version 2.3.4.3) with the command options: -k 4
261 --no-discordant. reads with more than 6 variants in 100 bases were discarded (SNV, deletion
262 or insertion each count as one variant). Variants were called using ivar variants (version
263 1.3.1). Consensus sequence was built based on the ivar variants table using the R Biostrings
264 package according to these rules: Positions with less than 10 reads were called as N.

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

273

274

275 Centre 15

276 Nucleic acids were extracted using the MagNA Pure 96 system and the DNA and viral RNA
277 small volume kit (Roche Diagnostics, Rotkreuz, Switzerland) or using the Abbott m2000
278 Realtime System and the Abbott sample preparation system reagent kit (Abbott, Baar,
279 Switzerland). Amplicon sequencing followed the ARTIC nCOV-2019 protocol with a weighted
280 v3 primer mix. Libraries were prepared with the Illumina DNA Prep kit (Illumina) on a
281 Hamilton STAR robot. Up to 96 samples were pooled equimolarly and sequenced paired-end
282 150bp on an Illumina NextSeq 500 mid output flow cell.

283

284 Reads were demultiplexed with bcl2fastq v.2.17 (Illumina) and assembled using the COVGAP
285 Pipeline (v10.6) (38) as previously described in (39,40). Briefly, a minimal depth of 50 was
286 required for bases to be called. SNPs were called with a minimum allele frequency of 0.7.
287 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.

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- 378

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.

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.

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.