

1 **Title**

2 *HaVoC*, a bioinformatic pipeline for reference-based consensus assembly and lineage  
3 assignment for SARS-CoV-2 sequences.

4

5 **Authors and institutional addresses**

6 Phuoc Truong Nguyen <sup>1</sup>, Ilya Plyusnin <sup>2,3</sup>, Tarja Sironen <sup>1,3</sup>, Olli Vapalahti <sup>1,3,4</sup>, Ravi Kant †<sup>1,3</sup>,  
7 Teemu Smura †<sup>1,4</sup>

8

9 1. *Department of Virology, Faculty of Medicine, University of Helsinki, Helsinki, Finland*

10 2. *Institute of Biotechnology, University of Helsinki, Helsinki, Finland*

11 3. *Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland*

12 4. *Department of Virology, University of Helsinki and Helsinki University Hospital, Helsinki,*  
13 *Finland*

14 †Correspondence to: [Ravi.Kant@helsinki.fi](mailto:Ravi.Kant@helsinki.fi) or [Teemu.Smura@helsinki.fi](mailto:Teemu.Smura@helsinki.fi)

15

16 **Abstract**

17 **Background:** SARS-CoV-2 related research has increased in importance worldwide since  
18 December 2019. Several new variants of SARS-CoV-2 have emerged globally, of which the  
19 most notable and concerning currently are the UK variant B.1.1.7, the South African variant  
20 B1.351 and the Brazilian variant P.1. Detecting and monitoring novel variants is essential in  
21 SARS-CoV-2 surveillance. While there are several tools for assembling virus genomes and  
22 performing lineage analyses to investigate SARS-CoV-2, each is limited to performing singular  
23 or a few functions separately.

24

25 **Results:** Due to the lack of publicly available pipelines, which could perform fast reference-  
26 based assemblies on raw SARS-CoV-2 sequences in addition to identifying lineages to detect

27 variants of concern, we have developed an open source bioinformatic pipeline called *HaVoC*  
28 (Helsinki university Analyzer for Variants Of Concern). *HaVoC* can reference assemble raw  
29 sequence reads and assign the corresponding lineages to SARS-CoV-2 sequences.

30

31 **Conclusions:** *HaVoC* is a pipeline utilizing several bioinformatic tools to perform multiple  
32 necessary analyses for investigating genetic variance among SARS-CoV-2 samples. The  
33 pipeline is particularly useful for those who need a more accessible and fast tool to detect and  
34 monitor the spread of SARS-CoV-2 variants of concern during local outbreaks. *HaVoC* is  
35 currently being used in Finland for monitoring the spread of SARS-CoV-2 variants. *HaVoC* user  
36 manual and source code are available at <https://www.helsinki.fi/en/projects/havoc> and  
37 [https://bitbucket.org/auto\\_cov\\_pipeline/havoc](https://bitbucket.org/auto_cov_pipeline/havoc), respectively.

38

### 39 **Keywords**

40 SARS-CoV2, variant detection, reference assembly, lineage identification, coronavirus,  
41 sequence analysis.

42

### 43 **Background**

44 Emerging pathogens pose a continuous threat to mankind, as exemplified by the Ebola virus  
45 epidemic in West Africa in 2014 [1], Zika virus pandemic in 2015 [2], and the ongoing  
46 Coronavirus disease 2019 (COVID-19) pandemic. These viruses are zoonotic, i.e. have crossed  
47 species barriers from animals to humans, alike the majority of emerging human pathogens [3,  
48 4]. The likelihood of this host switching is enhanced by several factors, e.g. global movement of  
49 people and animals, environmental changes, increased proximity of humans, wildlife and  
50 livestock, and population expansion into new environments [5].

51

52 The mutation and evolution rate of RNA viruses is considerably higher than their hosts, which is  
53 advantageous for viral adaptation. Mutations in the viral genome are most of the time silent or, if  
54 affecting phenotype, related to attenuation, although mutations can also lead to more  
55 pathogenic strains. A new virus variant may have one or more mutations that separate it from  
56 the wild-type virus already circulating among the general population.

57  
58 Coronaviruses (family *Coronaviridae*) are enveloped single-stranded RNA viruses, which cause  
59 respiratory, enteric, hepatic, and neurological diseases of a broad spectrum of severity among  
60 different animals and humans. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-  
61 2), a novel evolutionary divergent virus responsible for the present pandemic, has devastated  
62 societies and economies globally. The SARS-CoV-2 pandemic has already infected more than  
63 100 million people in 221 countries, causing over 2.2 million global deaths as of 3<sup>rd</sup> February  
64 2021 [6]. In autumn 2020, a new variant of SARS-CoV-2 known as 20B/501Y.V1 (B.1.1.7) was  
65 detected in south-eastern England, Wales, and Scotland [7]. This variant has since spread  
66 globally to more than 80 countries. The variant has undergone 23 mutations with 13-  
67 nonsynonymous mutations, four amino acid deletions, and six synonymous mutations making  
68 the virus more transmissible [8]. Another variant 20C/501Y.V2 (B.1.351) was detected in South  
69 Africa which was genetically distant from the UK 20B/501Y.V1 variant [9]. This South African  
70 variant with its two mutations in the receptor-binding motif that mainly forms the interface with  
71 the human ACE2 receptor has also been widely spreading to circulate globally. It has been  
72 noticed that some existing vaccines against SARS-CoV-2 are less effective against the  
73 20C/501Y.V2 variant [10–12]. A third variant being closely monitored is P.1 detected first in  
74 Brazil [13]. Interestingly, all these three variants have a mutation in the receptor binding domain  
75 (RBD) of the spike protein at position 501, where the amino acid asparagine (N) has been  
76 replaced with tyrosine (Y) enabling specific PCR to detect the N501Y mutation [14].

77

78 As more transmissible coronavirus variants are circulating worldwide, the role of researchers  
79 and technology specialists in controlling the pandemic has received more emphasis. The  
80 surveillance of virus variants by sequencing the SARS-CoV-2 genomes would provide a fast  
81 way to monitor variants and their spread, however, there are only few publicly available  
82 methods for quick reference-based consensus assembly and lineage assignment for SARS-  
83 CoV-2 samples. For this purpose, we have developed a simple pipeline, called *HaVoC* (Helsinki  
84 university Analyzer for Variants Of Concern), for quick reference-based consensus assembly  
85 and lineage assignment for SARS-CoV-2 samples. This will provide the end user a quick and  
86 accessible method of variant identification and monitoring. The pipeline was developed to be  
87 run on Unix/Linux operating systems, and thus can also be used in remote servers, e.g. CSC –  
88 IT Center for Science, Finland.

89

## 90 **Implementation**

91 *HaVoC* consists of a single shell script, which performs reference-based consensus assemblies  
92 to query SARS-CoV-2 fastq sequence libraries and assigns lineages to them individually in  
93 succession. The script can be started by typing the following line into your command line  
94 terminal:

95

```
96 sh HaVoC.sh [FASTQ directory]
```

97

98 The computing of consensus sequences starts with the tool detecting FASTQ files generated  
99 via paired end sequencing in a given input directory and checking that each query FASTQ file  
100 has its corresponding counterpart, i.e. mates file. The names of the files are modified to be more  
101 concise, e.g. Query-Seq:1\_X123\_Y000\_R1\_000.fastq.gz to Query-Seq:1\_R1.fastq.gz. The  
102 pipeline accepts FASTQ files both in gzipped and uncompressed format.

103

104 For the analyses, the user can choose which bioinformatic tools to utilize. This can be done by  
105 typing the tool wanted (*tools\_prepro*, *tools\_aligner* and *tools\_sam*) within the options section in  
106 the beginning of the script file. For example, if the user wants to deploy Trimmomatic to pre-  
107 process FASTQ files, the following line can be changed as follows:

108

109 From

110 *tools\_prepro="fastp"*

111 To

112 *tools\_prepro="trimmomatic"*

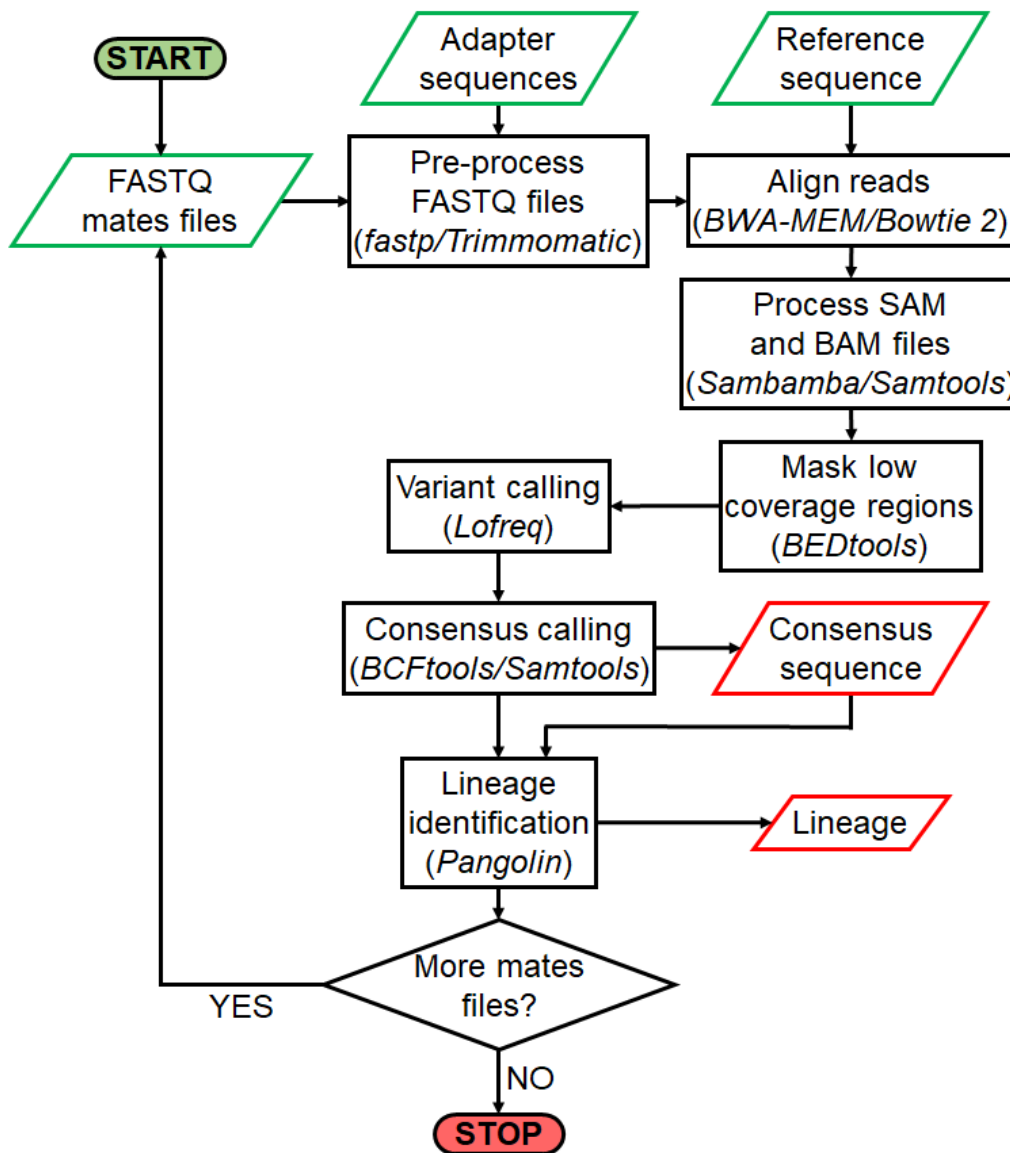
113

114 Other options include the number of threads, minimum coverage below which a region is  
115 masked (*min\_coverage*), and whether to run Pangolin to assign lineages to the consensus  
116 genome (*run\_pangolin*). An additional option allows *HaVoC* to be run in the CSC servers  
117 (*run\_in\_csc*).

118

119 The pre-alignment quality control, e.g. removing and trimming low quality reads and bases,  
120 removing adapter sequences, can be done with either fastp [15] or Trimmomatic [16]. The reads  
121 are then aligned to a reference genome of SARS-CoV-2 isolate Wuhan-Hu-1 (Genbank  
122 accession code: NC\_045512.2) with BWA-MEM [17] or Bowtie 2 [18]. The resulting SAM and  
123 BAM files are processed (includes sorting, filling in mate coordinates, marking duplicate  
124 alignments, and indexing reads) with Sambamba [19] or Samtools [20] and the low coverage  
125 regions are masked with BEDtools [21]. After masking a variant call is done with Lofreq [22]  
126 before computing the consensus sequence via BCFtools of Samtools [20]. Finally, the  
127 consensus sequence is analyzed with pangolin [23] to assign a lineage. The whole process is  
128 depicted in figure 1.

129



130

131 **Fig. 1** Flowchart describing processes and steps performed by *HaVoC* pipeline. The pipeline  
132 constructs consensus sequences from all FASTQ files in an input directory and then compares  
133 the resulting sequences to other established SARS-CoV-2 genomes to assign them the most  
134 likely lineages. The pipeline requires a FASTA file of adapter sequences for FASTQ pre-  
135 processing and a reference genome of SARS-CoV-2 in a separate FASTA file. The adapter file  
136 is not required when running the pipeline with fastp option. Input files are highlighted in green  
137 and the outputs in red.

138

139 Usage example

140 We are going to demonstrate a common use case for *HaVoC* with FASTQ files containing reads  
141 for SARS-CoV-2 sequences, provided by the Viral zoonoses research unit at University of  
142 Helsinki, Finland. The test files within the Example\_FASTQs folder contain paired-end FASTQ  
143 files for the UK variant (UK-variant-1) and the South African variant (S-Africa-variant-1). To  
144 analyse these example files, the aforementioned command needs to be deployed as follows:

145

146 `sh HaVoC.sh Example_FASTQs`

147

148 **Results**

149 The FASTQ files are processed and analyzed with the default options utilizing faster  
150 bioinformatic tools (fastp, BWA-MEM and Sambamba) in ca. 2–4 minutes, depending on the  
151 performance of the platform (local or server). After *HaVoc* has finished the analyses, each  
152 FASTQ file is moved to their respective result folders within the FASTQ directory. Each result  
153 folder contains a FASTA file for the consensus sequence (e.g. UK-variant-1\_consensus.fa) and  
154 a CSV file with the lineage information produced by pangolin (e.g. UK-variant-  
155 1\_pangolin\_lineage.csv). In addition to these main result files, each directory contains the  
156 original FASTQ files, BAM files (original, indexed and sorted), variant call files (VCF) with  
157 mutation data, BED file used for masking regions, and fastp report files with the results of  
158 FASTQ processing. The resulting directory and file structure with the example files will look as  
159 follows:

160 `Example_FASTQs/`

161 `UK-variant-1/`

162 `UK-variant-1.bam`

163 `UK-variant-1_R1.fastq.gz`

164 `UK-variant-1_R2.fastq.gz`

165 UK-variant-1\_consensus.fa  
166 UK-variant-1\_fixmate.bam  
167 UK-variant-1\_indel.bam  
168 UK-variant-1\_indel.vcf  
169 UK-variant-1\_indelflt.vcf  
170 UK-variant-1\_lowcovmask.bed  
171 UK-variant-1\_markdup.bam  
172 UK-variant-1\_namesort.bam  
173 UK-variant-1\_pangolin\_lineage.csv  
174 UK-variant-1\_sorted.bam  
175 fastp.html  
176 fastp.json  
177 S-Africa-variant-1/  
178 S-Africa-variant-1.bam  
179 S-Africa-variant-1\_R1.fastq.gz  
180 S-Africa-variant-1\_R2.fastq.gz  
181 S-Africa-variant-1\_consensus.fa  
182 S-Africa-variant-1\_fixmate.bam  
183 S-Africa-variant-1\_indel.bam  
184 S-Africa-variant-1\_indel.vcf  
185 S-Africa-variant-1\_indelflt.vcf  
186 S-Africa-variant-1\_lowcovmask.bed  
187 S-Africa-variant-1\_markdup.bam  
188 S-Africa-variant-1\_namesort.bam  
189 S-Africa-variant-1\_pangolin\_lineage.csv  
190 S-Africa-variant-1\_sorted.bam



191 fastp.html

192 fastp.json

193

194 Each of the example UK variants should have been categorized as B.1.1.7 and the South

195 African variants as B.1.351 (with pangoleARN release 2021-02-06). It is important to note

196 however, that as more sequences are uploaded and the pangolin lineage nomenclature

197 updated, the assigned lineages may differ from the expected ones described in this paper.

198 Regions with low coverages (with default setting under 30) are marked with the letter N during

199 masking and represent gaps in the final consensus sequences.

200

201 *HaVoC* is comparable to alternative combinations of tools, e.g. Jovian and pangolin, in both

202 speed and accuracy. These tools however operate separately, and as of publishing, there are

203 no single public tools that can both perform a reference-based consensus assembly and a

204 lineage identification in an easily accessible manner.

205

## 206 **Conclusions**

207 Early detection and understanding of the potential impact of emerging variants of SARS-CoV-2

208 is of primary importance and can assist in more efficient surveillance and control of the disease.

209 The likelihood of emergence of novel SARS-CoV-2 variants of concern is increased and

210 accelerated by the high mutation rates typical in RNA viruses and the growing number of

211 transmissions and infections both locally and globally.

212

213 With the rising number of variants detected worldwide and with many of them associated with

214 increased transmissibility and lower vaccine efficacy, there is an emerging need for fast,

215 efficient and reliable pipelines to help detect, identify and trace SARS-CoV-2 lineages. These

216 pipelines should in addition be accessible to researchers who may not be familiar with utilizing  
217 complex bioinformatic tools or scripting pipelines.

218

219 Due to these challenges, we have developed *HaVoC*, a simple, reliable and user-friendly  
220 pipeline, which can be simply downloaded from our repository and run without being installed.  
221 All its dependencies can be installed via existing package managers, of which we recommend  
222 Bioconda. *HaVoC* could help in the current pandemic situation by detecting variants of concern  
223 in the sequencing centers and public health or other organisations currently running and tracing  
224 variants of concern worldwide. *HaVoC* is currently utilized for detecting and tracing SARS-CoV-  
225 2 variants of concern, mainly B.1.1.7, B1.351 and P.1, in Finland.

226

## 227 **Availability and requirements**

228 *Project name:* *HaVoC* (Helsinki university Analyzer for Variants Of Concern)

229 *Project home page:* <https://www.helsinki.fi/en/projects/havoc> and

230 [https://bitbucket.org/auto\\_cov\\_pipeline/havoc](https://bitbucket.org/auto_cov_pipeline/havoc)

231 *Operating system(s):* Linux, Mac

232 *Programming language:* Shell script

233 *Other requirements:* Trimmomatic or Fastp, BWA-MEM or Bowtie2, Samtools, BEDtools,

234 BCFtools, Lowfreq and Pangolin.

235 *License:* GNU GPL

236 *Any restrictions to use by non-academics:* license needed

237

## 238 **List of abbreviations**

239 SARS-CoV-2 - Severe acute respiratory syndrome coronavirus 2

240 COVID-19 - Coronavirus disease 2019

241 *HaVoC* - Helsinki university Analyzer for Variants Of Concern

242

243 **References**

- 244 1. Dixon MG, Schafer IJ, Centers for Disease Control and Prevention (CDC). Ebola viral  
245 disease outbreak--West Africa, 2014. *MMWR Morb Mortal Wkly Rep.* 2014;63:548–51.
- 246 2. Kindhauser MK, Allen T, Frank V, Santhana RS, Dye C. Zika: the origin and spread of a  
247 mosquito-borne virus. *Bull World Health Organ.* 2016;94:675-686C.  
248 doi:10.2471/BLT.16.171082.
- 249 3. Taylor LH, Latham SM, Woolhouse ME. Risk factors for human disease emergence. *Philos*  
250 *Trans R Soc Lond B Biol Sci.* 2001;356:983–9. doi:10.1098/rstb.2001.0888.
- 251 4. Woolhouse MEJ, Gowtage-Sequeria S. Host range and emerging and reemerging  
252 pathogens. *Emerging Infect Dis.* 2005;11:1842–7. doi:10.3201/eid1112.050997.
- 253 5. Morens DM, Fauci AS. Emerging Pandemic Diseases: How We Got to COVID-19. *Cell.*  
254 2020;182:1077–92. doi:10.1016/j.cell.2020.08.021.
- 255 6. Worldometer - COVID-19 Virus Pandemic. <https://www.worldometers.info/coronavirus/>.  
256 Accessed 3 Feb 2021.
- 257 7. Rambaut A, Loman N, Pybus O, Barclay W, Barrett J, Carabelli A, et al. Preliminary genomic  
258 characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike  
259 mutations. *Virological.* 2020. [https://virological.org/t/preliminary-genomic-characterisation-of-an-](https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563)  
260 [emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563](https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563).  
261 Accessed 2 Feb 2021.
- 262 8. Leung K, Shum MH, Leung GM, Lam TT, Wu JT. Early transmissibility assessment of the  
263 N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. *Euro*  
264 *Surveill.* 2021;26. doi:10.2807/1560-7917.ES.2020.26.1.2002106.
- 265 9. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Emergence  
266 and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-  
267 CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv.* 2020.

- 268 doi:10.1101/2020.12.21.20248640.
- 269 10. Mahase E. Covid-19: Novavax vaccine efficacy is 86% against UK variant and 60% against  
270 South African variant. *BMJ*. 2021;:n296. doi:10.1136/bmj.n296.
- 271 11. Kupferschmidt K. Vaccine 2.0: Moderna and other companies plan tweaks that would  
272 protect against new coronavirus mutations. *Science*. 2021. doi:10.1126/science.abg7691.
- 273 12. Edwards E. J&J says vaccine effective against Covid, though weaker against South Africa  
274 variant. *NBC News*. 2021. [https://www.nbcnews.com/health/health-news/j-j-vaccine-effective-](https://www.nbcnews.com/health/health-news/j-j-vaccine-effective-against-covid-though-weaker-against-south-n1255400)  
275 [against-covid-though-weaker-against-south-n1255400](https://www.nbcnews.com/health/health-news/j-j-vaccine-effective-against-covid-though-weaker-against-south-n1255400). Accessed 10 Feb 2021.
- 276 13. Faria NR, Claro IM, Candido D, Franco LAM, Andrade PS, Coletti TM, et al. Genomic  
277 characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings.  
278 *Virological*. 2021. [https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-](https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586)  
279 [lineage-in-manaus-preliminary-findings/586](https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586). Accessed 3 Feb 2021.
- 280 14. Centers for Disease Control and Prevention (CDC). Emerging SARS-CoV-2 Variants.  
281 [https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-](https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html)  
282 [emerging-variants.html](https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html). Accessed 12 Feb 2021.
- 283 15. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor.  
284 *Bioinformatics*. 2018;34:i884–90. doi:10.1093/bioinformatics/bty560.
- 285 16. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data.  
286 *Bioinformatics*. 2014;30:2114–20. doi:10.1093/bioinformatics/btu170.
- 287 17. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.  
288 arXiv. 2013.
- 289 18. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*.  
290 2012;9:357–9. doi:10.1038/nmeth.1923.
- 291 19. Tarasov A, Vilella AJ, Cuppen E, Nijman IJ, Prins P. Sambamba: fast processing of NGS  
292 alignment formats. *Bioinformatics*. 2015;31:2032–4. doi:10.1093/bioinformatics/btv098.
- 293 20. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence

- 294 Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25:2078–9.  
295 doi:10.1093/bioinformatics/btp352.
- 296 21. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features.  
297 *Bioinformatics*. 2010;26:841–2. doi:10.1093/bioinformatics/btq033.
- 298 22. Wilm A, Aw PPK, Bertrand D, Yeo GHT, Ong SH, Wong CH, et al. LoFreq: a sequence-  
299 quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from  
300 high-throughput sequencing datasets. *Nucleic Acids Res*. 2012;40:11189–201.  
301 doi:10.1093/nar/gks918.
- 302 23. pangolin. <https://github.com/cov-lineages/pangolin>. Accessed 12 Feb 2021.

303

#### 304 **Declarations**

305 Ethics approval and consent to participate

306 Not Applicable.

307

308 Consent for publication

309 Not Applicable.

310

311 Availability of data and materials

312 Publicly available at [https://bitbucket.org/auto\\_cov\\_pipeline/havoc](https://bitbucket.org/auto_cov_pipeline/havoc).

313

314 Competing interests

315 The authors declare that they have no competing interests.

316

317 Funding

318 This study was supported by the Academy of Finland (grant number 336490), VEO - European  
319 Union's Horizon 2020 (grant number 874735) and the Jane and Aatos Erkko Foundation.

320

321 Authors' contributions

322 Conceptualization: PTN IP RK TS TSi OV. Development: PTN IP RK TS. Testing/Formal

323 analysis: PTN IP RK TS. Funding acquisition: TSi OV. Investigation: PTN IP RK TS.

324 Methodology: PTN IP RK TS. Project administration: RK TS OV. Resources: PTN RK IP TS TSi

325 OV. Validation: PTN IP RK TS. Writing – original draft: PTN RK. Writing – review & editing: IP

326 TS TSi OV.

327

328 Acknowledgements

329 None.

330

331 Authors' information

332 None.