

1 **Title**

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

4

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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, 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 3rd 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"*

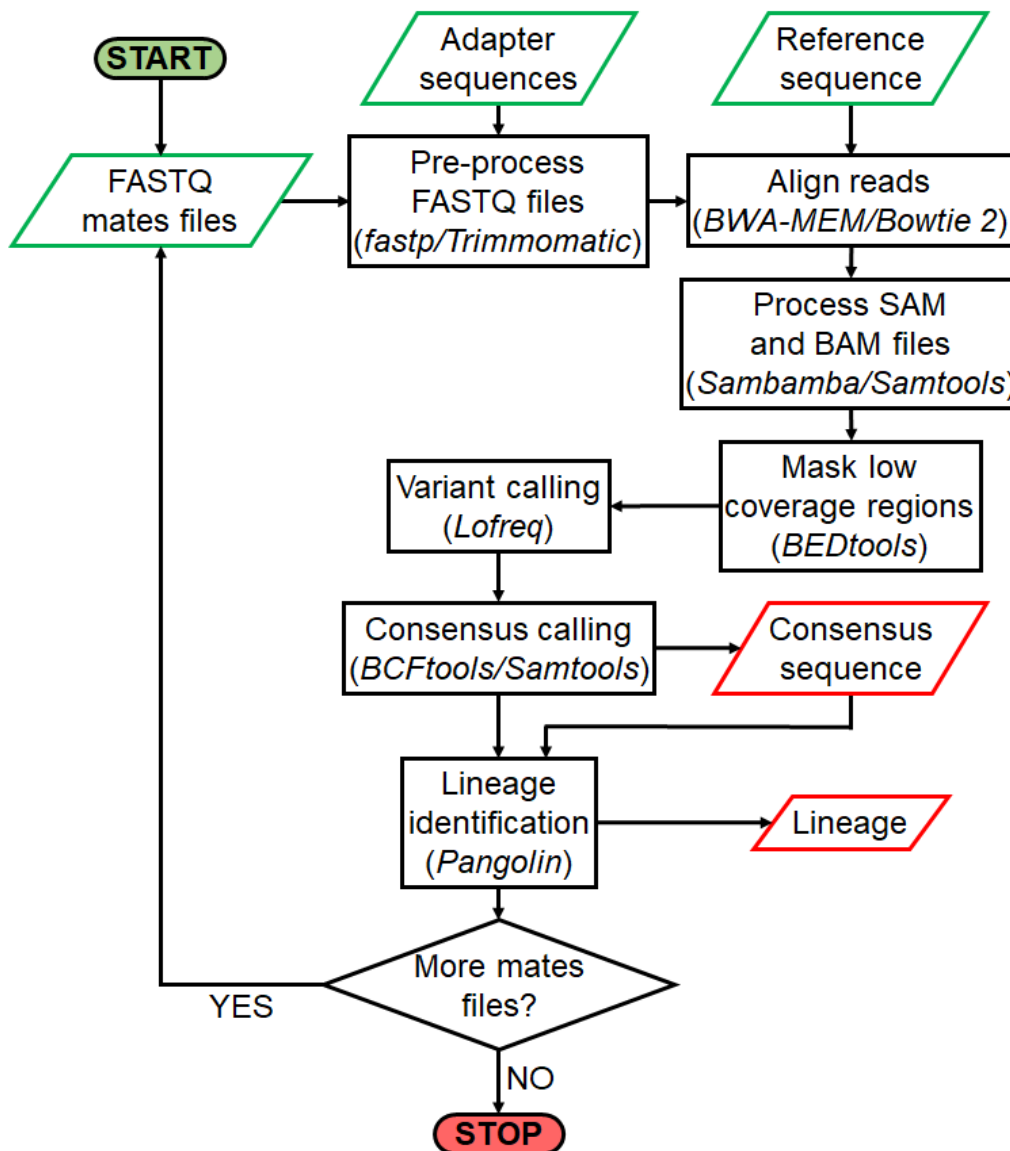
113

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

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

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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).
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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.

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.