InterARTIC: an interactive web application for whole-genome nanopore sequencing analysis of SARS-CoV-2 and other viruses

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1. ABSTRACT

Motivation: *InterARTIC* is an interactive web application for the analysis of viral whole-genome sequencing (WGS) data generated on Oxford Nanopore Technologies (ONT) devices. A graphical interface enables users with no bioinformatics expertise to analyse WGS experiments and reconstruct consensus genome sequences from individual isolates of viruses, such as SARS-CoV-2. *InterARTIC* is intended to facilitate widespread adoption and standardisation of ONT sequencing for viral surveillance and molecular epidemiology.

Worked example: We demonstrate the use of *InterARTIC* for the analysis of ONT viral WGS data from SARS-CoV-2 and Ebola virus, using a laptop computer or the internal computer on an ONT GridION sequencing device. We showcase the intuitive graphical interface, workflow customization capabilities and job-scheduling system that facilitate execution of small- and large-scale WGS projects on any virus.

Implementation & availability: *InterARTIC* is a free, open-source web application implemented in Python. The application can be downloaded as a set of pre-compiled binaries that are compatible with all common Ubuntu distributions or built from source. For further details please visit: https://github.com/Psy-Fer/interARTIC/.

2. INTRODUCTION

Viral whole-genome sequencing (WGS) has become a critical tool used to guide local, national and international public health responses to the ongoing COVID-19 pandemic, as well as Ebola, Zika and other previous epidemics (Eden *et al.*, 2020) (Fauver *et al.*, 2020) (Gonzalez-Reiche *et al.*, 2020) (Gudbjartsson *et al.*, 2020) (Lu *et al.*, 2020) (Meredith *et al.*, 2020) (Msomi *et al.*, 2020) (Rockett *et al.*, 2020) (du Plessis *et al.*, 2021) (Quick *et al.*, 2016, 2017). Viral WGS is used to define the phylogenetic structure of disease outbreaks in order to better understand geographical spread, resolve transmission networks and infer the origin of unknown cases (Eden *et al.*, 2020) (Fauver *et al.*, 2020) (Gonzalez-Reiche *et al.*, 2020) (Rockett *et al.*, 2020). Viral WGS can also identify novel strains, and monitor virus evolution over time or in response to public health interventions, such as vaccines (du Plessis *et al.*, 2021) (Fiorentini *et al.*, 2021) (Tegally *et al.*, 2021) (Williams and Burgers, 2021).

Oxford Nanopore Technologies (ONT) is emerging as a leading sequencing platform for viral WGS. ONT devices are cheap, require minimal supporting laboratory infrastructure or

technical expertise for sample preparation, and can be used to perform rapid viral WGS with high consensus-level accuracy (Bull *et al.*, 2020). ONT's pocket-sized MinION device can be used to perform portable viral sequencing in the field or the clinic (Quick *et al.*, 2016, 2017) (Samarakoon *et al.*, 2020). Meanwhile, ONT's benchtop GridION and PromethION devices enable high-throughput sequencing of large sample cohorts, with internal computers that can be utilised for data processing.

The ARTIC network for viral surveillance and molecular epidemiology has been instrumental in driving the adoption of ONT sequencing for viral WGS. ARTIC is an international consortium that has developed standardised protocols for WGS analysis of multiple common viruses, including SARS-CoV-2. ARTIC researchers have also developed best-practice, open-source bioinformatics workflows for the reconstruction of consensus viral genome sequences from ONT sequencing data. While these command-line driven workflows are a critical resource for the community, they require specialist bioinformatics expertise that is not available to many research and public health laboratories, particularly in under-resourced areas. Moreover, the dependency of ARTIC pipelines on multiple third-party software packages creates difficulties during installation and can prevent version standardisation between different labs/sites.

To address these issues, we have developed *InterARTIC*, an interactive local web application for the analysis of viral WGS data generated on ONT devices. *InterARTIC* incorporates best-practice bioinformatics workflows for viral WGS analysis into an intuitive graphical interface that allows novice users to reconstruct consensus genome sequences from individual viral isolates, including SARS-CoV-2. The workflows are fully customisable, ensuring compatibility with different viruses and/or upstream laboratory preparations and an intelligent job-scheduling system facilitates efficient handling of large projects. We provide *InterARTIC* as a free, open-source tool to facilitate further adoption and standardisation of ONT sequencing for viral surveillance during the ongoing COVID-19 pandemic and future disease outbreaks.

3. IMPLEMENTATION & AVAILABILITY

The central component of *InterARTIC* is a simple and intuitive graphical user interface developed using Python, HTML, CSS and JavaScript that encapsulates current best-practice command-line bioinformatics pipelines from ARTIC (**Fig.1A**). *InterARTIC* uses Flask as the web framework and features an asynchronous task queue implemented using Celery with Redis as the database backend. *InterARTIC* is provided as a standalone package that bundles the aforementioned graphical interface, the ARTIC pipeline (fieldbioinformatics toolkit) and all the dependencies.

InterARTIC requires no installation of third-party software and itself can be downloaded as a set of pre-compiled binaries that has been tested with Ubuntu 14, 16, 18, 20 (supports both native Ubuntu as well as Ubuntu installed on Windows via windows subsystem for linux). Advanced users can alternatively build *InterARTIC* from source.

The application can execute all analysis on a standard laptop or desktop PC, after receiving sequencing data from an ONT device, such as a MinION. Alternatively, *InterARTIC* can run the analysis on the internal computer of a GridION/PromethION benchtop sequencer, generating all outputs without the need for data transfer.

4. OVERVIEW

4.1. Launching the application

To use *InterARTIC*, the binaries should be downloaded, extracted and launched by running the following simple commands in a bash terminal:

```
$ wget
https://github.com/Psy-Fer/interARTIC/releases/download/v0.2-beta/interartic-v0.2-l
inux-x86-64-binaries.tar.gz -O interartic_bin.tar.gz
$ tar xf interartic_bin.tar.gz
$ cd interartic_bin
$ ./run.sh
```

This launches an interactive session that can be accessed through a standard web browser, via a link that is printed to the standard output (by default: http://127.0.0.1:5000), as follows:

```
$ ./run.sh

InterARTIC is now running on your machine :)
To see your InterARTIC session and launch a job, please visit:
http://127.0.0.1:5000
Keep this terminal open to keep your InterARTIC session active.
To terminate the session type CTRL-C or close the terminal.
```

After navigating to the URL, the user can interact with the graphical interface in order to set up and launch their analysis (**Fig.1B,C**).

4.2. Summary of the workflow.

InterARTIC analyses amplicon-based viral WGS data generated on ONT devices. It is compatible with any virus genome, and any standard or custom amplicon primer scheme. *InterARTIC* inputs base-called sequencing reads (FASTQ format), which are typically generated during a sequencing run, using the *Guppy* base-caller within ONT's *MinKNOW* sequencing manager software (**Fig. 1Ai**). If multiple samples were sequenced on a single flow cell, *InterARTIC* uses *Porechop* to demultiplex the library into individual sample barcodes, then runs each sample separately through the analytic workflow (**Fig. 1Aii)**.

InterARTIC first uses *Minimap2* to align sequencing reads to a viral reference genome (e.g., MN908947 for SARS-CoV-2; **Fig. 1Aiii**). Primer sequences are then trimmed, and alignments are down-sampled to a maximum coverage-depth threshold that can be specified by the user (**Fig. 1Aiv**). The genome (FASTA format) and primer site (BED format) reference files used during these steps can be selected from a range of popular options or supplied as custom files, according to the user's needs. Genetic variants (SNVs and indels) are then identified relative to the reference genome. *InterARTIC* currently supports two alternative workflow choices for variant detection that utilise the popular tools *Nanopolish* or *Medaka*/*Longshot*, with the user selecting either or both workflows from a dropdown menu (**Fig. 1Av**). Low-quality variant candidates are then excluded (**Fig. 1Avi**) and regions of low coverage are masked from the reference genome (**Fig. 1Avii**). Finally, variant candidates are incorporated into the masked reference genome using *Bcftools* to generate a consensus genome sequence for the viral isolate under analysis (**Fig. 1Aviii**). This consensus genome sequence is suitable for downstream applications including lineage classification and

phylogeographic analysis (**Fig. 1Aix**), and ready for upload to public repositories (e.g., GISAID).

In addition to the detected variants (VCF format) and consensus genome (FASTA format) output files from the workflow, *InterARTIC* also generates and displays several informative summaries and data plots that facilitate quick inspection of the data (**Fig. 1D,E**).

InterARTIC employs an asynchronous job queue system with Celery to allow multiple jobs of the same, or differing virus and/or amplicon scheme, to be launched to allow for efficient use of compute resources (**Fig. 1B**). This is especially important if running analyses on a GridION/PromethION benchtop device, alongside other sequencing jobs.

4.3. Example data.

To demonstrate the use of *InterARTIC*, we analysed publicly available nanopore WGS datasets from SARS-CoV-2 (10 x isolates, sequenced on an ONT GridION) and Ebola (2 x isolates, sequenced on an ONT MinION). Example screenshots of the workflow configuration process for each experiment are provided in **Supplementary Figures 1 & 2, a**nd example output files generated by *InterARTIC* are also available for download.

Both projects were analysed using *InterARTIC* on a standard laptop PC, and separately on a GridION sequencing device **Supplementary Table 1**. Indicative run-time statistics on both devices are reported in **Supplementary Table 2**. We encourage users to download these example datasets to test *InterARTIC* on their own machine.

5. ACKNOWLEDGMENTS

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6. DECLARATIONS

J.M.F. and H.G. have received travel and accommodation expenses to speak at Oxford Nanopore Technologies conferences. The authors declare no other competing financial or non-financial interests.

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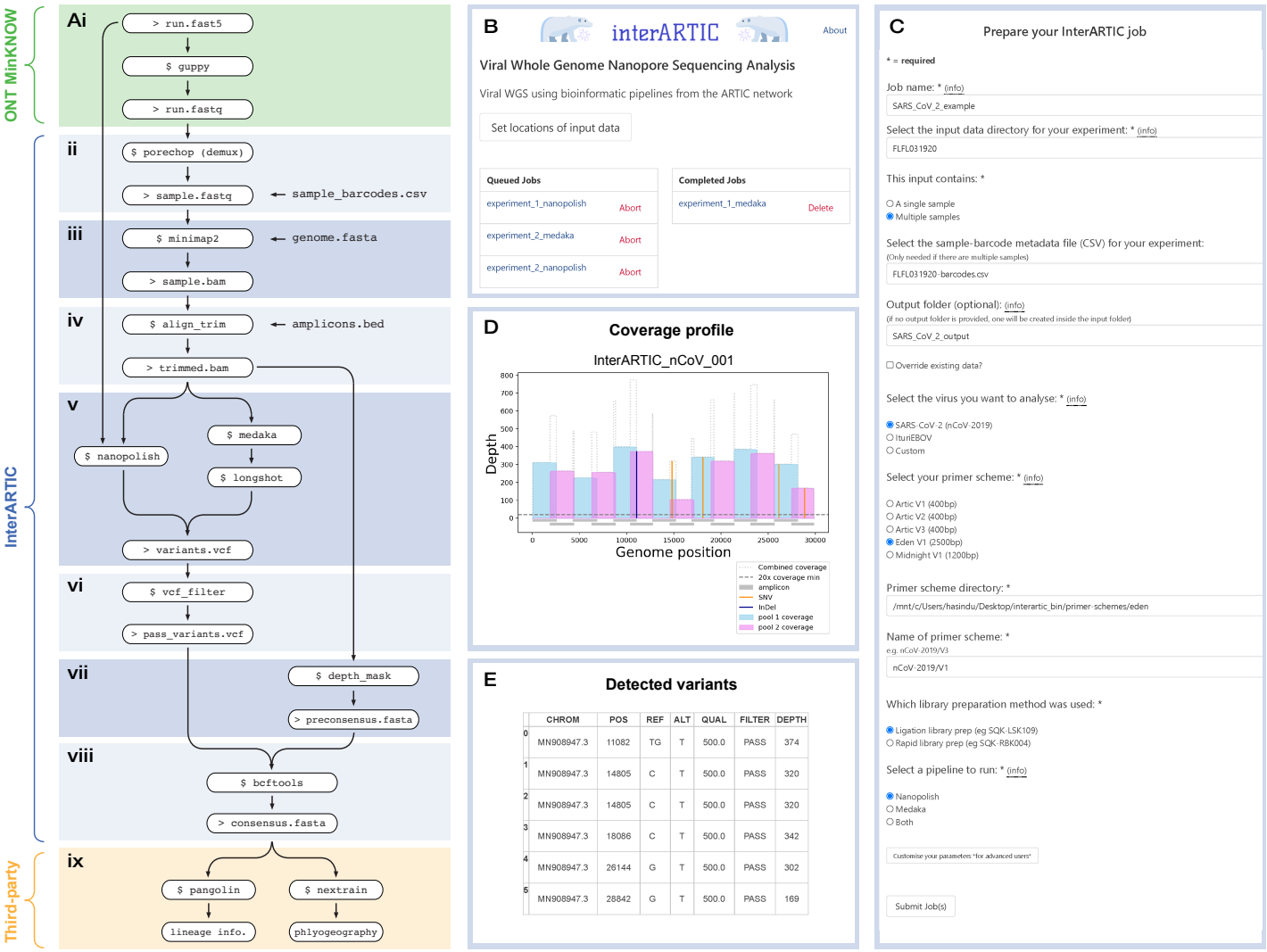

	CHROM	POS	REF	ALT	QUAL	FILTER	DEPTH
0	MN908947.3	11082	TG	T	500.0	PASS	374
1	MN908947.3	14805	C	T	500.0	PASS	320
2	MN908947.3	14805	C	T	500.0	PASS	320
3	MN908947.3	18086	C	T	500.0	PASS	342
4	MN908947.3	26144	G	T	500.0	PASS	302
5	MN908947.3	28842	G	T	500.0	PASS	169

Figure 1. Overview of the *InterARTIC* workflow and graphical interface. (**A**) Schematic summary of a typical workflow for the analysis of ONT viral genome sequencing data. Workflow steps ii-viii (blue tiles) are executed by *InterARTIC*. (**B**) Example screenshot of the *InterARTIC* interface hompage, with queued and completed jobs displayed. (**C**) Example of the project setup page within the interface, where the user may customise their workflow. Note: full parameter selection options for advanced users are hidden. (**D**) Example of a coverage profile plot for SARS-CoV-2 that is generated and displayed by *InterARTIC*. (**E**) Example of the variant detection table that is generated and displayed.

	Laptop	GridION
Model	Dell XPS	GridION Mk1
Processor	Intel i7-8750H	Intel i7-7700
RAM	16 GB	64 GB
Disk	1TB NVMe SSD	4TB NVMe SSD (RAID configuration)
O/S	Ubuntu 16.04.7 LTS on WSL for Windows 10	Ubuntu 16.04.7 LTS
Threads used	4	4

Supplementary Table 1. Specifications of computers used to run example workflows.

					Laptop run-time (min:sec)				GridION run-time (min:sec)			
Set	**Virus**	**Amplicons**	**Samples**	**Pipeline**	**Total**	**Gather**	**Demux**	**Analysis**	**Total**	**Gather**	**Demux**	**Analysis**
1	SARS-CoV-2	Eden V1 2.5kb	10	Nanopolish	32:19	00:07	17:56	14:05	25:09	00:05	13:42	11:33
				Medaka-Longshot	30:24	0:11	17:53	12:09	21:41	00:05	13:43	07:45
3	Ebola	Artic V1 400bp	2	Nanopolish	03:57	00:02	02:23	01:30	02:55	00:01	01:42	01:10
				Medaka-Longshot	04:09	00:03	02:24	01:42	02:54	00:01	01:40	01:12

Supplementary Table 2. Indicative run-times for *InterARTIC* workflow on example viral WGS datasets.