SARS-CoV-2 RECoVERY: a multi-platform open-source bioinformatic pipeline for the automatic construction and analysis of SARS-CoV-2 genomes from NGS sequencing data

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Abstract

Background: Since its first appearance in December 2019, the novel *Severe Acute Respiratory Syndrome Coronavirus type 2* (SARS-CoV-2), spread worldwide causing an increasing number of cases and deaths (35,537,491 and 1,042,798, respectively at the time of writing, https://covid19.who.int). Similarly, the number of complete viral genome sequences produced by Next Generation Sequencing (NGS), increased exponentially. NGS enables a rapid accumulation of a large number of sequences. However, bioinformatics analyses are critical and require combined approaches for data analysis, which can be challenging for non-bioinformaticians.
Results: A user-friendly and sequencing platform-independent bioinformatics pipeline, named SARS-CoV-2 RECoVERY (REconstruction of CoronaVirus gEnomes & Rapid analYsis) has been developed to build SARS-CoV-2 complete genomes from raw sequencing reads and to investigate variants. The genomes built by SARS-CoV-2 RECoVERY were compared with those obtained using other software available and revealed comparable or better performances of SARS–CoV2 RECoVERY. Depending on the number of reads, the complete genome reconstruction and variants analysis can be achieved in less than one hour.
The pipeline was implemented in the multi-usage open-source Galaxy platform allowing an easy access to the software and providing computational and storage resources to the community.
Conclusions: SARS-CoV-2 RECoVERY is a piece of software destined to the scientific community working on SARS-CoV-2 phylogeny and molecular characterisation, providing a performant tool for the complete reconstruction and variants' analysis of the viral genome. Additionally, the simple software interface and the ability to use it through a Galaxy instance without the need to implement computing and storage infrastructures, make SARS-CoV-2 RECoVERY a resource also for virologists with little or no bioinformatics skills.

Availability and implementation: The pipeline SARS-CoV-2 RECoVERY (REconstruction of COronaVirus gEnomes & Rapid analYsis) is implemented in the Galaxy instance ARIES (https://aries.iss.it).

Introduction

On December 2019, a novel coronavirus was reported in patients with pneumonia infections in Wuhan, China (Zhu et al., 2020). The novel coronavirus, *Severe Acute Respiratory Syndrome Coronavirus type 2* (SARS-CoV-2), and the related disease, *Coronavirus Disease 2019* (COVID-19) (Gorbalenya et al., 2020), spread rapidly culminating in the WHO declaration of the pandemic state on March 2020, which is still ongoing.

During the pandemic outbreak, NGS technologies are allowing complete genome sequencing of thousands of viral strains worldwide and the assessment of temporal and geographical virus spreading (e.g., EpiCOV/GISAID: https://www.gisaid.org).

The NGS technologies produce millions of sequences, however, the manipulation and processing of files can be challenging due to files' size and can be affected by the lack of bioinformatic skills. The different sequencing standards available (Ambardar et al., 2016) (e.g. Illumina, Ion Torrent, Nanopore) are supported by platforms developed by the companies and made available for the users to a certain extent. On the other hand, the scientific community frequently performs analysis of sequencing data, through commercial software, requiring payment of license keys, or in-house command-line-based pipelines, which imply the availability of bioinformatic skills. In this study, with the intention to provide an all-in-one tool aimed at SARS-CoV2 genomes reconstruction and analysis we concatenated common command-line-based tool into a pipeline, named SARS-CoV-2 RECoVERY (REconstruction of COronaVirus gEnomes & Rapid analYsis), implemented on the multi-usage open-source Galaxy instance ARIES (https://aries.iss.it), dedicated to public health microbiology (Knijn et al., 2020).

Methods

Overview

The SARS-CoV-2 RECoVERY consists of seven steps: (1) read quality analysis and trimming, (2) subtraction of human sequences, (3) reads alignment and reference mapping against the SARS-CoV-

2 reference sequence, (4) variant calling, (5) consensus sequence calling, (6) *de novo* assembly, (7) ORFs identification and variant annotation.

Building databases

The GenBank file of the reference genome of SARS-CoV-2 (isolate Wuhan-Hu-1; Accession number: NC045512.2) was used to build two databases: a fasta format file containing the complete virus genome used as reference and a database contaning the Open Reading Frames (ORF) annotation by the SnpEff tool (Cingolani et al., 2012) in gbk format.

Read quality analysis and trimming

The reads imported in fastq format are trimmed with the Trimmomatic tool (Bolger et al., 2014) to remove the low-quality bases (or N bases) from both terminus of each read and to exclude reads shorter than 30 base pairs (bp).

Subtraction of human sequences

Trimmed reads are mapped using Bowtie2 software (Langmead et al., 2012) onto the reference human genome downloaded by "The Genome Reference Consortium" database (https://www.ncbi.nlm.nih.gov/grc) to remove the human genomic sequences

Genome reconstruction

The recovered unaligned reads are mapped onto the reference sequence of SARS-CoV-2 using the software Bowtie2, for Illumina and Ion Torrent reads, and Minimap2 (Li, 2018) for Nanopore reads. The output BAM file is processed using the mpileup tool and bcftools from SAMtools (Li et al., 2009) to call SNPs and indels. The complete genome sequence is constructed using an in-house modified version of bcftools consensus. This tool inserts an "N" in each nucleotide position not covered by sequencing, or where the coverage is lower than 30 repetitions (30x).

Coverage analysis

The coverage analysis and nucleotide distribution are performed using the tool Qualimap 2 (Okonechnikov et al., 2016).

Contig assembling

Contigs are generated through *de novo* assembly performed by the software Spades with default parameters (Bankevich et al., 2012).

ORF annotation

Annotation is performed with the BLASTn tool (Megablast) using the SARS-CoV-2 reference ORFs (Open Reading Frame). Because of the high nucleotide identities among SARS-CoV-2 strains, >99% nucleotide identity has been set as a requirement for the ORFs annotation. The parameters used for the alignments are: 1 as maximum number of hits, 80% identity cut-off, and 80% Minimum query coverage per High-scoring Segment Pair (HSP). The output table is converted in a multi-fasta file containing the ORFs identified.

Variant annotation

The SnpEff tool is used for variant annotation using the reference genome of SARS-CoV-2. The tabular output contains: the variant position on the genome, the nucleotide of the reference and the alternative sequence, the codon of the reference and the alternative codon, the nucleotide translation and the information about the mutation (synonymous or missense).

Performance of the pipeline in comparison with other software

One hundred NGS raw data from Illumina, 100 from Nanopore and 50 from Ion Torrent platforms, were downloaded from the NCBI database Sequence Read Archive (SRA). The SARS-CoV-2 genomes from the Ion Torrent and Illumina raw data were built using the pipeline from this study, the CLC Genomics Workbench Ver. 9.5 (Qiagen, Milano, Italy) and the online tool Genome Detective Virus Tool (Vilsker et al., 2019). The Nanopore raw data were analysed only by Genome Detective and our pipeline, since CLC does not accept long reads as input. Finally, the genomes reconstructed from each SRA using the different software, were compared to the corresponding GISAID sequence used as reference. We recorded differences between reconstructed genomes in terms of length difference in comparison with the GISAID reference sequences and number of different nucleotides called, calculated by arithmetic mean.

Results and Discussion

In this study, we describe the development of a pipeline for the construction and analysis of SARS-CoV-2 genomes and the comparison of the results with those obtained by CLC Genomics Workbench 9.5, Genome Detective Virus Tool using the GISAID sequences as a reference. The SRA used for the analyses were obtained using Illumina, IonTorrent and Nanopore as sequencing standards and corresponded to the GISAID entries used as reference and downloaded from NCBI database. Most of the genomes built using our pipeline were longer (54 nucleotides on average) than the corresponding GISAID references and those built by CLC and Genome Detective for all the sequencing standards (Table 1). In detail, 96% (48/50) of Ion Torrent, 73% (73/100) of Illumina and 97% (97/100) of Nanopore raw reads produced longer genomes when our pipeline was used. Additionally, these genomes presented less nucleotide differences ($n \leq 7$, mean) than the genomes built with other software when compared to the GISAID sequence used as reference.

This finding is of particular interest as such differences may include either incorrect or missing nucleotide assignment, which would hamper the studies on SARS-CoV2 evolution and distribution, since the mutations described so far in SARS-CoV-2 genomes are mainly single point mutations. Since the discovery of the SARS-CoV-2 and the first complete genome sequencing (Wu et al., 2020), 378.326 genomes have been submitted to the GISAID database allowing the prompt identification of mutations, together with geographical and temporal mapping of the circulating strains. Besides Whole Genome Sequencing, bioinformatics analyses are pivotal to obtain the final results.

The pipeline developed in this study is publicly accessible through the Galaxy instance ARIES (https://aries.iss.it) and provides a user-friendly interface, allowing the complete reconstruction of SARS-CoV-2 genomes in 10 to 60 minutes for NGS data composed by 50 thousand to 6 million reads. The analyses can be run independently from the users' hardware and the software can be accessed upon direct registration on the ARIES home page using any browser running on desktop or mobile devices. In addition, ARIES does not request access to the users' data but rather provides a

service to the scientific community to boost the knowledge on the evolution of the SARS-CoV-2 in the attempt to favour a global response to this global threat.

We developed an all-in-one and sequencing platform-independent pipeline for the complete SARS-CoV-2 genome reconstruction and analysis to support the scientific community in the analysis of such data.

The comparison of the genomes obtained with the pipeline and those obtained using some of the software available showed comparable or better performances of the former solution. The simplicity of use and the production of a comprehensive report with all the variations characterized, make this pipeline a valuable tool particularly for scientists with little or no skill in bioinformatic.

Conclusions

In conclusion, we developed a pipeline for the complete genome reconstruction and analysis of sequence data to help and speed the scientific community in the analysis of SARS-CoV-2 data and to share the same method. The analyses have been completely automated, and the user interface has been designed to minimize the input from the user in order to provide a support also for the non-bioinformaticians and to enlarge the base of scientists contributing data.

The release of the software as an open-source pipeline through a Galaxy instance will also allow the scientific community to use this collaborative platform in a reproducible way for the crowdsourcing-based advance of our understanding of this new virus and the different evolutionary scenarios.

Authors' contributions

All authors contributed to writing the paper, LDS and GI tested the software, AK and SM developed and designed the software, GV, IDB and SM conceived of the project idea and provided advice and assistance throughout the development of the software and the manuscript writing process.

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Table 1. Results of comparison of GISAID reference genome and those built by CLC, Genome detective and the SARS-CoV-2 RECoVERY.

GISAID	Mean difference* in consensus length	Min-Max of difference in consensus length		% of consensus sequences longer then GISAID reference	% of consensus sequences with different nucleotide call	Mean** of n° of different nucleotide call
Ion Torrent data (n° of analysed runs =50)						
CLC	-137	-544	+47	2 (4%)	28 (56%)	4
SARS-CoV-2 RECoVERY	+54	-2	+106	48 (96%)	48 (93.7%)	7
Genome Detective	-5172	-18454	+11	0 (0%)	49 (99.9%)	41
Illumina data (n° of analysed runs =100)						
CLC	-1173	-8345	+643	18 (18%)	20 (20%)	7
SARS-CoV-2 RECoVERY	+135	-1652	+3379	73 (73%)	52 (52%)	5
Genome Detective	-167	-8925	+1989	40 (40%)	43 (43%)	16
Nanopore data (n° of analysed runs =100)						
SARS-CoV-2 RECoVERY	+569	-169	+2444	97 (97%)	96 (96%)	7
Genome Detective	+267	-3816	+2127	91 (91%)	90 (90%)	13

* Difference in consensus length: calculated as the arithmetic mean of the differences between the corresponding genome built by each software and the GISAID reference length.

** Different nucleotide call: calculated as the arithmetic mean of the number of different sites (nucleotides) between the GISAID reference and the corresponding genome built by each software.