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CoV-Seq: SARS-CoV-2 Genome Analysis and Visualization

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Abstract

Summary: COVID-19 has become a global pandemic not long after its inception in late 2019. SARS-CoV-2 genomes are being sequenced and shared on public repositories at a fast pace. To keep up with these updates, scientists need to frequently refresh and reclean datasets, which is ad hoc and labor-intensive. Further, scientists with limited bioinformatics or programming knowledge may find it difficult to analyze SARS-CoV-2 genomes. In order to address these challenges, we developed CoV-Seq, a webserver to enable simple and rapid analysis of SARS-CoV-2 genomes. Given a new sequence, CoV-Seq automatically predicts gene boundaries and identifies genetic variants, which are presented in an interactive genome visualizer and are downloadable for further analysis. A command-line interface is also available for high-throughput processing.
Availability and Implementation: CoV-Seq is implemented in Python and Javascript. The webserver is available at `http://covseq.baidu.com/` and the source code is available from `https://github.com/boxiangliu/covseq`.
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Supplementary information: Supplementary information are available at *bioRxiv* online.

1 Introduction

The 2019 Novel Coronavirus (SARS-CoV-2) caused an outbreak of viral pneumonia since December of 2019 and have since become a global pandemic. Despite efforts to contain its spread, SARS-CoV-2 has infected more than 3 millions patients and caused more than 200,000 deaths worldwide as of late April (Dong *et al.*, 2020). To understand its evolution and gene functions, scientists have sequenced SARS-CoV-2 genomes from patients across different age groups, genders, ethnicities, locations, and disease stages (Hadfield *et al.*, 2018). These genomic sequences are being shared on public repositories at a rapid pace, with thousands of new sequences every week (Elbe and Buckland-Merrett, 2017; Brister *et al.*, 2015; Kanz *et al.*, 2005; Wei, 2019). To keep up with the latest developments, scientists need to frequently download and reclean new datasets, which is ad hoc and time-consuming. On the other hand, scientists with limited knowledge in bioinformatics or programming may experience difficulty in analyzing SARS-CoV-2 genomes.

We developed the CoV-Seq toolkit to address these challenges. CoV-Seq consists of several components: a data analysis pipeline that takes FASTA sequences and generates variant callsets in VCF format and open reading frame (ORF) predictions. The pipeline automatically filters low-quality sequences and removes duplicate sequences, performs sequence alignment, as well as identifies and annotates genetic variants (Figure 1A). We provide a webserver (`http://covseq.baidu.com/`) to allow rapid analysis of custom sequences without any programming. The web interface includes an interactive genome visualizer and tabulated displays of genetic variants and ORF predictions. All results can be downloaded for downstream analysis. Further, we provide a command-line interface to allow high-thoughput processing on local environments. To facilitate data sharing, we aggregate SARS-CoV-2 sequences from GISAID (Elbe and Buckland-Merrett, 2017), NCBI (Brister *et al.*, 2015), EMBL (Kanz *et al.*, 2005) and CNGB (Wei, 2019), and publish annotated variant callsets and metadata on a weekly basis.

2 Analysis and Visualization of Viral Genomes

The collection of SARS-CoV-2 genomic sequences is rapidly expanding. An integrated pipeline is essential for keeping current with frequent updates. To understand the evolution and the functional genome of a new viral strain, it is necessary to identify its genetic mutations and gene boundaries. Existing software packages such as VAPiD (Shean *et al.*, 2019) and VIGOR (Wang *et al.*, 2010) focus on gene annotation. To our knowledge, a software package to identify, annotate and visualize genetic variants for SARS-CoV-2 is lacking.

CoV-Seq provides an intuitive web interface for analyzing and visualizing SARS-CoV-2 variants and ORFs. Upon receiving custom sequences, CoV-Seq identifies gene boundaries and genetic variants and displays them alongside a full-length genome (Figure 1B). Users interact with the genome by dragging the zoom bar to adjust the magnification and the position bar to pan along the genome. When the genome window contains less than 150 nucleotides, letters will appear to indicate both the nucleotide bases and amino acid residues. Hovering over gene bodies or variants will trigger a pop-up window for relevant information. Clicking an ORF brings up three tables. A first table to show all variants and their positions, alleles, and ORFs in which they belong (Figure 1C). A second table shows ORF annotations obtained by aligning input sequences against the reference sequence and transferring annotations from Genbank (Figure 1D). A third table shows nucleotide and protein sequences for the selected ORF. All tables can be downloaded for further analysis.

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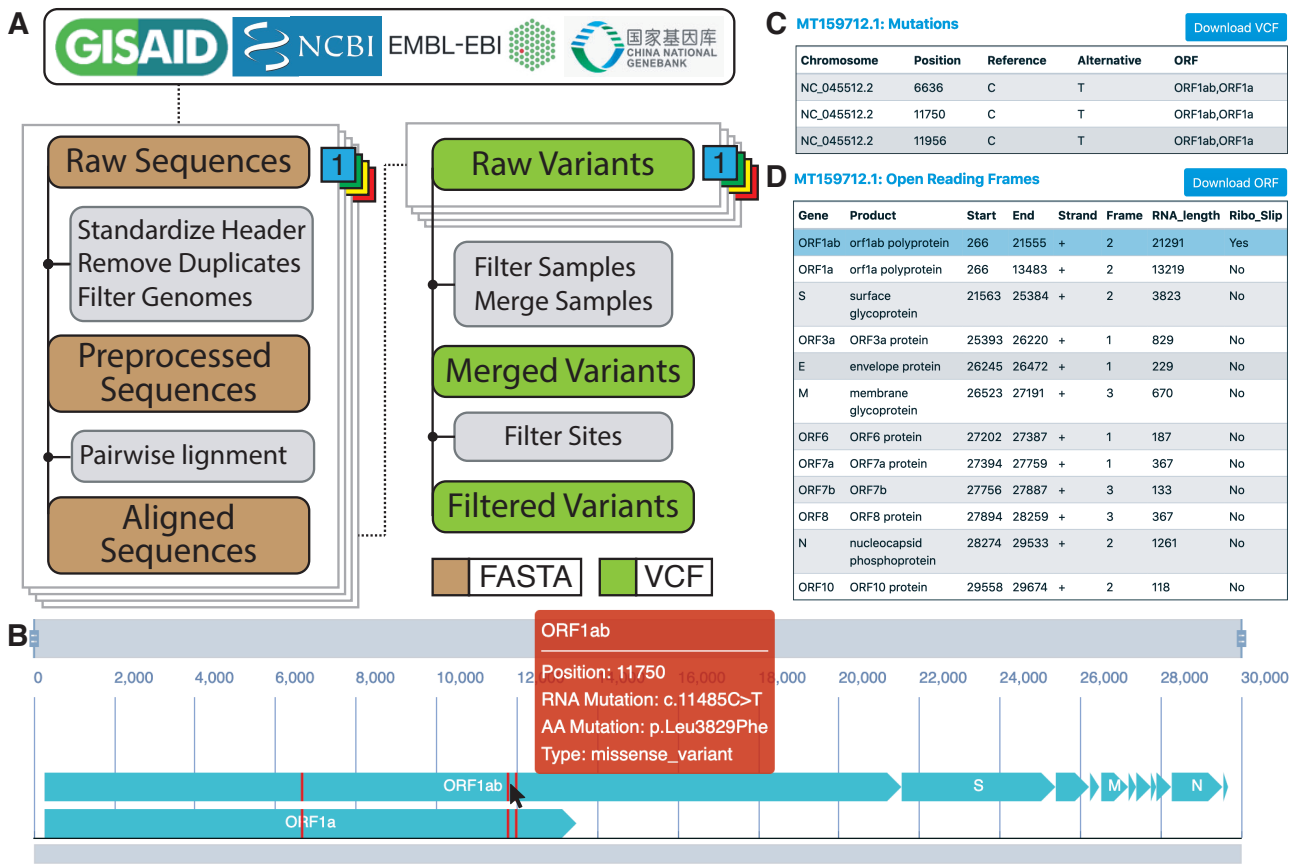

Chromosome	Position	Reference	Alternative	ORF
NC_045512.2	6636	C	T	ORF1ab,ORF1a
NC_045512.2	11750	C	T	ORF1ab,ORF1a
NC_045512.2	11956	C	T	ORF1ab,ORF1a

Gene	Product	Start	End	Strand	Frame	RNA_length	Ribo_Slip
ORF1ab	orf1ab polyprotein	266	21555	+	2	21291	Yes
ORF1a	orf1a polyprotein	266	13483	+	2	13219	No
S	surface glycoprotein	21563	25384	+	2	3823	No
ORF3a	ORF3a protein	25393	26220	+	1	829	No
E	envelope protein	26245	26472	+	1	229	No
M	membrane glycoprotein	26523	27191	+	3	670	No
ORF6	ORF6 protein	27202	27387	+	1	187	No
ORF7a	ORF7a protein	27394	27759	+	1	367	No
ORF7b	ORF7b	27756	27887	+	3	133	No
ORF8	ORF8 protein	27894	28259	+	3	367	No
N	nucleocapsid phosphoprotein	28274	29533	+	2	1261	No
ORF10	ORF10 protein	29558	29674	+	2	118	No

Fig. 1. The CoV-Seq pipeline and web interface. (A) Genomic sequences are collected from GISAID, NCBI, EMBL and CNGB. We remove incomplete genomes (length < 25,000) and duplicate genomes before alignment with MAFFT against the reference genome NC_045512.2. We use a custom Python script to generate raw variant calls and remove samples with too many mutations indicative of sequencing error. After merging VCFs, we remove multi-allelic sites and variants with the poly-A tail for a filtered set of variants. (B) The interactive genome visualizer shows ORFs (turquoise) and mutations (red). Users can zoom with the top bar and pan with the bottom bar. Hovering over gene bodies and mutations will trigger pop-up windows for relevant information. (C) The mutation table shows positions, alleles and intersecting ORFs. (D) The ORF table shows predicted gene boundaries and supporting information.

3 SARS-CoV-2 genome processing pipeline

Extraction of genetic variants from sequences involves the following steps (Figure 1): (1) preprocessing: removal of low-quality and duplicated sequences; (2) alignment: pairwise alignment of each sequence against a reference; (3) variant calling: identify differences between aligned sequences; and (4) postprocessing: remove low-quality sites and annotate variants.

We aggregate SARS-CoV-2 sequences from GISAID, NCBI, EMBL and CNGB, and remove sequences too short to be considered whole genomes. Some incomplete genomes contain a single gene while others contain large gaps. Such gaps may be indistinguishable from deletion variants and could give rise to false positive findings. We use a lenient cutoff of 25,000 nucleotide because it removes distinctly incomplete genomes while retains complete genomes (Figure S1). We append genome lengths to the metadata for users to set custom cutoffs. Both NCBI and EMBL are part of the International Nucleotide Sequence Database Collaboration (INSDC) and therefore contain duplicate submissions, which we remove by comparing the Accession IDs. Further, dual submissions can appear in both GISAID and INSDC under different Accession IDs. We consider two submissions as suspect duplications if they have identical genomic sequences. These suspect duplications are marked in the metadata but not removed because it is possible for an identical strain to infect multiple patients. We perform pairwise alignment against the reference sequence NC_045512.2 using MAFFT (Katoh and Standley, 2013) and use a custom Python script for variant calling. We left-normalize each variant with bcftools (Li, 2011) and remove samples with too many variants indicative of sequencing error. We use a lenient cutoff of 150 variants because it removes samples with extreme number of variants (>1,000) while keeping most samples (Figure S2). We append the number of variants to the metadata to allow users to set custom cutoffs. During postprocessing, we remove multi-allelic sites because these sites are more likely to occur in regions prone to sequencing error, such as the two ends of the genome (Figure S3). Further, we remove variants within the poly-A tail.

Both raw and filtered VCF files are available for download on CoV-Seq (`http://covseq.baidu.com/browse`) and the pipeline is open source at `https://github.com/boxiangliu/covseq`.

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References

Brister, J. R. *et al.* (2015). NCBI viral genomes resource. *Nucleic acids research*, **43**(D1), D571–D577.

Dong, E. *et al.* (2020). An interactive web-based dashboard to track COVID-19 in real time. *The Lancet infectious diseases*.

Elbe, S. and Buckland-Merrett, G. (2017). Data, disease and diplomacy: GISAID's innovative contribution to global health. *Global Challenges*, **1**(1), 33–46.

Hadfield, J. *et al.* (2018). Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics*, **34**(23), 4121–4123.

Kanz, C. *et al.* (2005). The EMBL nucleotide sequence database. *Nucleic acids research*, **33**(suppl_1), D29–D33.

Katoh, K. and Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution*, **30**(4), 772–780.

Li, H. (2011). A statistical framework for snp calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, **27**(21), 2987–2993.

Shean, R. C. *et al.* (2019). VAPiD: a lightweight cross-platform viral annotation pipeline and identification tool to facilitate virus genome submissions to ncbi genbank. *BMC bioinformatics*, **20**(1), 48.

Wang, S. *et al.* (2010). VIGOR, an annotation program for small viral genomes. *BMC bioinformatics*, **11**(1), 451.

Wei, X. (2019). China National GeneBank Database. *FAIRsharing.org*.