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CoronaSPAdes: from biosynthetic gene clusters to coronaviral assemblies

Dmitry Meleshko (d.meleshko@spbu.ru)[1,2], Anton Korobeynikov (a.korobeynikov@spbu.ru)[1,3*]

[1]Center for Algorithmic Biotechnology, Institute for Translational Biomedicine, St. Petersburg State University, St. Petersburg, Russia 199004;

[2]Tri-Institutional PhD Program in Computational Biology and Medicine, Weill Cornell Medical College, New York, New York 10021, USA;

[3]Department of Statistical Modelling, St. Petersburg State University, St. Petersburg, Russia 198504;

*Corresponding author

Abstract

COVID-19 pandemy has ignited a broad scientific interest in coronavirus research. The identification of coronaviridae species in natural reservoirs often requires de novo assembly. However, existing transcriptome assemblers often are not able to assemble coronaviruses into a single contig. We developed coronaSPAdes, a new module for SPAdes assembler for coronavirus species recovery. coronaSPAdes uses the knowledge about coronaviridae genome structure to improve assembly. We have shown that coronaSPAdes outperforms existing SPAdes modes and other popular short-read assemblers in the recovery of full-length coronavirus genomes. This should allow to better understand the coronaviridae spread and diversity.

Keywords

Genome assembly, transcriptome assembly, COVID-19, coronaviridae, hidden markov models

Background

COVID-19 pandemy has increased a scientific interest in coronavirus research. The analysis of coronavirus dataset starts with obtaining full-length virus genome sequence that can be performed using read alignment[1,2] or de novo assembly[1,3]. The assembly pipeline based on read alignment is a tool of choice for the same strains of the close species, e.g. for SARS-CoV-2 SNP profiling of confirmed COVID-19 patients. De novo assembly is better suited for novel species recovery since read alignment for distant species is unreliable. Recently, there were multiple studies that used MEGAHIT[4] assembler to recover full-length sequence of the SARS-CoV-2 genome. Though previous studies show that different SPAdes[5] modes also perform well in virus recovery[6]. Nevertheless, none of these assemblers was initially designed for viral assemblies in general and for coronaviridae species recovery in particular. MEGAHIT and metaSPAdes[7] are metagenomic assemblers, SPAdes[8] is designed to assemble single-cell and isolate bacterial datasets. All these assemblers can produce fragmented assemblies due to sequencing artifacts, coverage variations, host contamination, multiple strains presence, and coronavirus splice events[9]. Fast and correct characterization of virus datasets might be a key step in predicting and preventing the future outbreaks. Coronaviruses have a conserved gene structure[10] that can help to better assemble full-length genomes. In this study, we present coronaSPAdes - a new mode for SPAdes assembler designed to assemble coronaviridae species.

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coronaSPAdes borrows its algorithmic ideas from the existing SPAdes[11] modes (metaSPAdes[7], rnaSPAdes[12], metaviralSPAdes[13]), and includes HMM-guided assembly inspired by biosyntheticSPAdes[14]. We show that coronaSPAdes is able to recover novel full-length genomes from publicly available datasets where other popular assemblers produce fragmented assembly.

Results and Discussion

coronaSPAdes was used by the Serratus project [15] to assemble more than 10,000 putative coronaviral genomes out of 3.6 million SRA datasets. The Serratus benchmarking and results will be provided elsewhere. Here we highlight the features of coronaSPAdes using the range of publicly available transcriptome and metatranscriptome datasets that include novel and known species.

Fr4nk is a putative novel Alphocoronavirus detected in a metatranscriptome sequencing library from a Peruvian vampire bat (*Desmodus rotundus*, SRA:ERR2756788). Assembly of this sequencing library with coronaSPAdes yielded a 29264 nt viral genome. The average coverage is 110x with variation from 2x to 1500x in different regions of the genome. This is a new species of Coronavirus based on RdRP, nucleoprotein, membrane protein and replicase 1a, which all classify this virus an Alphacoronavirus outside of all named sub-genera and most similar to a Pedacovirus.

Ginger is a putative novel Alphacoronavirus detected in a transcriptome sequencing library from a Wildcat (*Felis silvestris*, SRA:SRR72871109). Assembly of this sequencing library with coronaSPAdes yielded a 29277 nt viral genome (see Fig. 1). The average coverage is 20x with variation from 230x to 6x in different parts of the genome.

[insert Figure 1 around here]

PEDV is a known Alphacoronavirus that causes porcine epidemic diarrhea. It was assembled from a transcriptome sequencing library of epithelial cells of pig intestine (*Sus scrofa*, SRA:SRR10829953). Assembly of this sequencing library with coronaSPAdes resulted in a 27973 nt viral genome. The average coverage is 470x with variation from 30x to 8000x in different parts of the genome.

We benchmark **Fr4nk**, **Ginger** and **PEDV** using several specialized virus assemblers (IVA, PRICE), generic metagenome and transcriptome assemblers (MEGAHIT, metaSPAdes, rnaSPAdes) and coronaSPAdes. The overview of the results could be found in Table 1. Conventional RNA assemblers (IVA and PRICE) are using a seed-and-extend approach, therefore a seed sequence was required. This property greatly reduces their applicability for nover species search. In addition, it seems they were unable to deal with the specifics of large transcriptome and metatranscriptome datasets. Other assemblers (MEGAHIT, metaSPAdes, rnaSPAdes) overall have shown acceptable results, however their performance was not uniform, as none of them was able to assemble complete virus genomes out of all 3 datasets. coronaSPAdes was able to produce whole genomes in all cases.

[insert Table 1 around here]

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Conclusions

In summary, we developed coronaSPAdes, a specialized assembler for coronaviridae genomes. coronaSPAdes allows to recover coronaviridae genomes with lower fragmentation and higher contiguity from the datasets of different nature. Moreover, our results prove the utility of a HMM-guided assembly approach that can be adapted to other genome types.

Methods

RNA virus assemblers [16], 17], 18] have to face a number of challenges in order to assemble the sequence data into a consensus sequence. These challenges stem from the nature of the sequencing data due to the biases in the reverse transcription and polymerase chain reaction amplification process that current sequencing methods rely on. These biases are further aggravated by enormous viral population diversity causing lots of SNPs as well as structural variations. These properties of the data cause assembly fragmentation or, even worse, make certain regions disappear from the assembly. Additionally, coronaviruses are known to use discontinuous extension of negative strands to produce multiple mRNAs [19]. So, even in case of a single virus in the sample, assemblers should be able to deal with multiple produced “isoforms”.

Over the years SPAdes team produced several assembly pipelines aimed for different kinds of sequencing data and tasks. This includes metaSPAdes for assembly of consensus genomes from metagenomes, rnaSPAdes centered around reconstruction of multiple isoforms from eukaryotic data as well as more specialized versions such as metaplasmidSPAdes and metaviralSPAdes. The latter pipeline uses coverage-based heuristics in order to detect putative DNA virus sequences (cyclic and linear) from assembly graphs.

It turned out that none of these pipelines could cope with all the challenges connected with RNA viral data, however, each of it contains some useful algorithms and approaches. The modular structure of SPAdes allowed deep reuse and extension of the existing algorithms to be combined into a coronaSPAdes pipeline.

Essentially, the coronaSPAdes pipeline consists of two main steps: rnaviralSPAdes and HMMPathExtension.

rnaviralSPAdes constructs assembly graph from input RNA viral dataset (transcriptome, meta-transcriptome, virome and meta-virome datasets are expected and supported). Briefly, it is based on metaSPAdes pipeline with several important additions and changes adopted from other SPAdes pipelines:

1. Removal of low-complexity (poly-A / poly-T) tips and edges and RNA-seq specific chimeric connections. rnaSPAdes introduced extensive procedures to remove from the de Bruijn graph artifacts that are specific to RNA data. As stated in [12] the majority of the chimeric connections in RNA-Seq data are either single-strand chimeric loops or double-strand hairpins. They are detected by analyzing the graph topology rather than nucleotide sequences or coverage. Another characteristic of RNA-Seq datasets is the large number of low-complexity regions that originate from poly-A tails resulting from polyadenylation at the ends of mRNAs. To avoid chimeric connections and non-informative sequences low-complexity edges are removed from the de Bruijn graph.
2. Removal of subspecies-bases variation. As rnaviralSPAdes like metaviralSPAdes aims for species-level assemblies (as opposed to strain-level assemblies that are certainly infeasible due to high level of variation), the bulge removal procedure was refined to collapse this variation. Specifically, it collapses long and similar (with respect to the edit distance) parallel edges in the assembly graph.
3. Additionally, we relaxed the metagenomic edge disconnector condition (see [7] for more info) due to coronavirus genome size and drastically increased coverage variation compared with metagenomic samples.

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rnaviralSPAdes pipeline allows for removal of the majority of RNA sequencing artifacts and collapsing the variation. However, still there are cases when an assembler could not remove the errors neither using the coverage-based heuristic nor the graph topology. The GINGER dataset outlined above is a good example. Certainly, it might be possible to tune various assembler heuristics to deal with this particular case, however, the solution will unlikely work on other datasets and would require extensive benchmarking in order not to be overly-aggressive in error elimination.

The second step of coronaSPAdes pipeline, HMMtraversal, deals with outlined problems in a completely different way and tries to use the information about coronaviral genome organization to distinguish between putative genomic sequences from uncleaned artifacts.

HMMPathExtension extends HMM-based algorithms of biosyntheticSPAdes. Similarly with biosyntheticSPAdes, HMMtraversal tries to find paths on the assembly graph that go through all significant HMM matches in order and agree with rnaviralSPAdes contigs. On contig breakpoints (e.g. in the case of remaining erroneous connections or variations), HMMtraversal is able to do a search for the nearest feasible match. This way the extracted genomic sequence is supported both by the graph topology and the structure of the genome.

coronaSPAdes is bundled with the Pfam SARS-CoV-2 set of HMMs [20]. Note that despite the name, these HMMs are quite general and represent the profiles of various proteins that belong to coronaviruses as well as more conserved ones like RdRp that is conserved across all RNA viruses [21]. Hits from these HMMs uniformly cover the genome of coronaviruses, allowing to reconstruct strains mixtures and splice variants.

We explicitly note that the approach of HMMPathExtension is not limited to coronaviral genomes. HMMPathExtension step allows for custom HMM database specification effectively enabling HMM-guided assemblies of other genomes using their internal structure.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Serratus data, including Frank and Ginger datasets are publically available. Access instructions can be found at https://github.com/ababaian/serratus/wiki/Access-Data-Release. coronaSPAdes version used in this article is available at http://cab.spbu.ru/software/coronaspades/. coronaSPAdes will be included in the future SPAdes releases.

Competing interests

The authors declare that they have no competing interests

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Authors contribution

DM came up with an algorithmic solution. DM and AK developed the software, analyzed the data, wrote and revised the manuscript. The authors read and approved the final manuscript.

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(Vancouver style)

		MEGAHIT	metaSPAdes	rnaSPAdes	coronaSPAdes	IVA	PRICE
Fr4nk	Longest	**29219**	26321	20607	**29164**	N/A**,^	8504*
	CheckV completeness	105.2	94.85	72.48	101.7	N/A	31.1
	CheckV AA avg ID%	55.71	56.13	63.34	54.84	N/A	75.8
Ginger	Longest	23905	19453	**29301**	**29277**	3186**	15691*
	CheckV completeness	91.1	69.0	103.66	103.5	N/A	55.9
	CheckV AA avg ID%	85.3	93.93	85.58	85.58	N/A	95.73
PEDV	Longest	**28530**	26316	23312	**27973**	N/A**,^	23559*

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	CheckV completeness	101.4	93.6	82.93	99.5	N/A	83.02
	CheckV AA avg ID%	97.55	97.96	97.92	97.5	N/A	97.9

Table 1: Benchmarking of assemblers on several datasets. Shown are: longest viral contig assembled, its completeness as estimated by CheckV[22] and the average amino acid identity to the closest reference in CheckV database. The best results are shown in bold.
*Seed was required, 1 kbp of coronaSPAdes assembly was used
**IVA failed to select seed automatically. 1 kbp of coronaSPAdes assembly was provided as a seed
^Failed to extend the seed

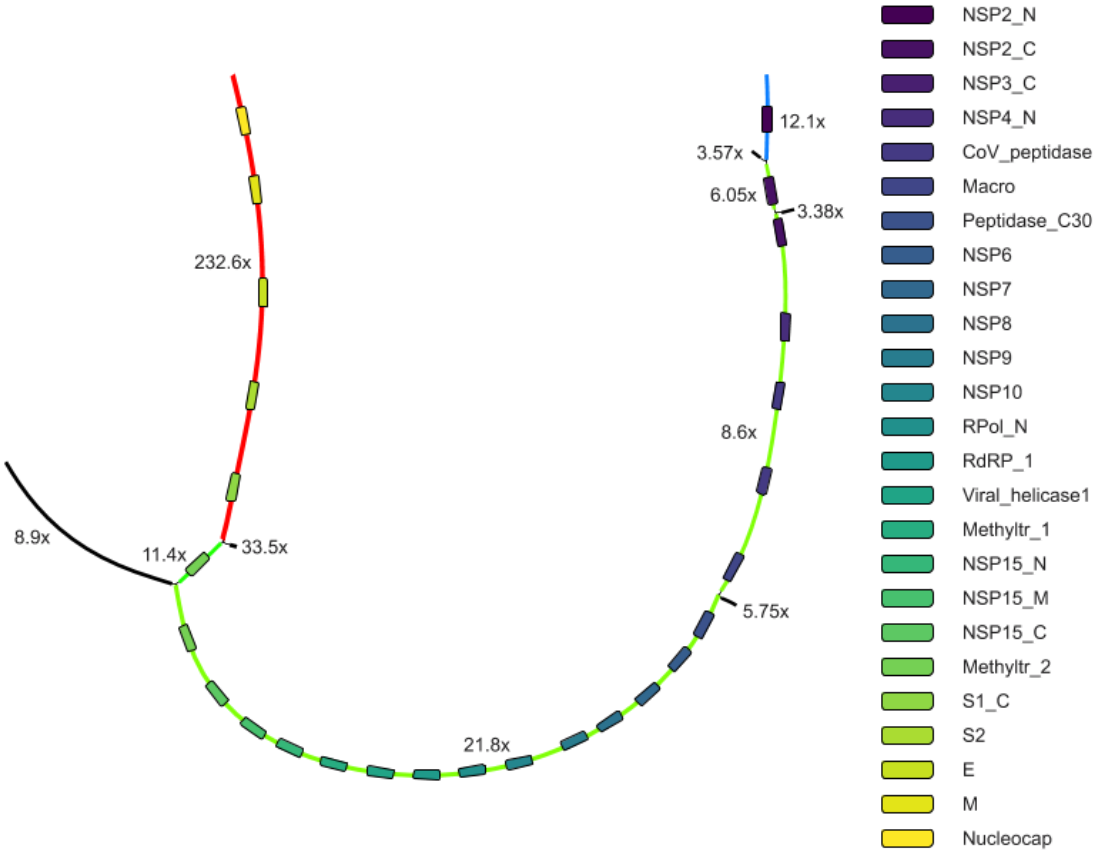
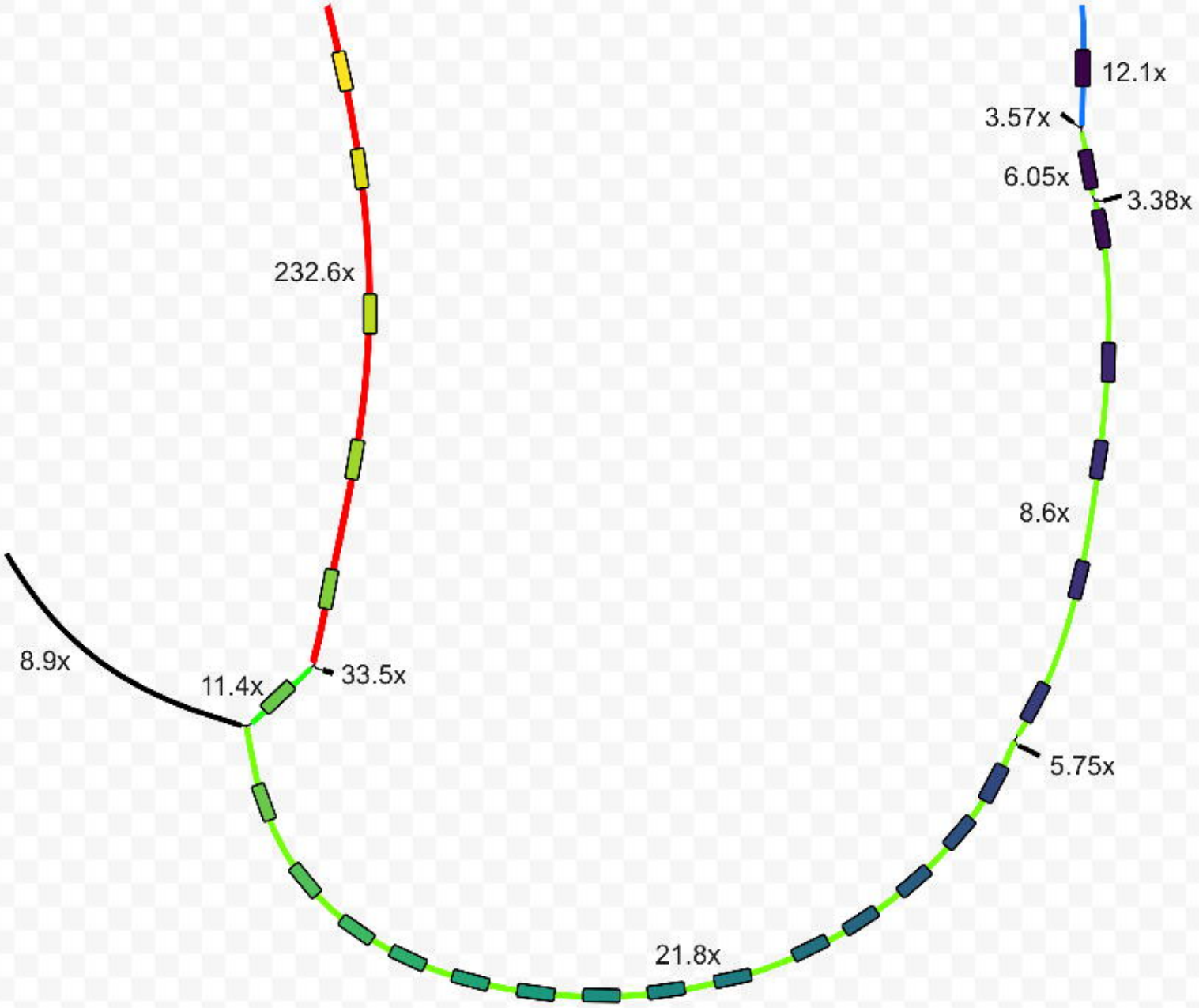


Figure 1: Part of Ginger assembly graph produced with coronaSPAdes. rnaviralSPAdes produced 8 contigs from this subgraph (red, green and blue paths on the graph and 5 black edges), therefore splitting the coronavirus genome into three parts. coronaSPAdes matched viral edges of the graph with domain (rectangles of different color). Path along these matches spells a full-length viral genome.



NSP2_N
NSP2_C
NSP3_C
NSP4_N
CoV_peptidase
Macro
Peptidase_C30
NSP6
NSP7
NSP8
NSP9
NSP10
RPol_N
RdRP_1
Viral_helicase1
Methyltr_1
NSP15_N
NSP15_M
NSP15_C
Methyltr_2
S1_C
S2
E
M
Nucleocap