Bioinformatics, 2024, **40(11)**, btae609
https://doi.org/10.1093/bioinformatics/btae609
Advance Access Publication Date: 14 October 2024
Applications Note

OXFORD

Genome analysis

Cluster-efficient pangenome graph construction with nf-core/pangenome

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Associate Editor: Can Alkan

Abstract

Motivation: Pangenome graphs offer a comprehensive way of capturing genomic variability across multiple genomes. However, current construction methods often introduce biases, excluding complex sequences or relying on references. The PanGenome Graph Builder (PGGB) addresses these issues. To date, though, there is no state-of-the-art pipeline allowing for easy deployment, efficient and dynamic use of available resources, and scalable usage at the same time.

Results: To overcome these limitations, we present *nf-core/pangenome*, a reference-unbiased approach implemented in Nextflow following nf-core's best practices. Leveraging biocontainers ensures portability and seamless deployment in High-Performance Computing (HPC) environments. Unlike PGGB, nf-core/pangenome distributes alignments across cluster nodes, enabling scalability. Demonstrating its efficiency, we constructed pangenome graphs for 1000 human chromosome 19 haplotypes and 2146 *Escherichia coli* sequences, achieving a two to threefold speedup compared to PGGB without increasing greenhouse gas emissions.

Availability and implementation: nf-core/pangenome is released under the MIT open-source license, available on GitHub and Zenodo, with documentation accessible at https://nf-co.re/pangenome/docs/usage.

1 Introduction

The availability of high-quality population-wide whole-genome assemblies (Liu *et al.* 2020, Leonard *et al.* 2022, Zhou *et al.* 2022, Kang *et al.* 2023, Liao *et al.* 2023, Weller *et al.* 2023) offers new opportunities to study sequence evolution and variation within and between genomic populations. A challenge is simultaneously representing and analyzing hundreds to thousands of genomes at a gigabase scale. One solution here is a pangenome. It models a population's entire set of genomic sequences (Ballouz *et al.* 2019). In contrast to reference-based genomic approaches, which relate sequences to a linear genome, pangenomics relates each new sequence to all the others represented in the pangenome (The Computational Pan-Genomics Consortium 2018, Eizenga *et al.* 2020, Sherman and Salzberg 2020) minimizing reference-bias. Pangenomes can be described as sequence graphs which store DNA sequences in nodes with edges connecting the nodes as they occur in the individual sequences (Hein 1989). Genomes are encoded as paths traversing the nodes (Garrison *et al.* 2018).

Current pangenome graph construction methods exclude complex sequences or are reference-biased (Minkin *et al.* 2017, Chin *et al.* 2023). One recent approach that overcomes such limitations is the PanGenome Graph Builder (PGGB) pipeline (Garrison *et al.* 2024). PGGB iteratively refines an all-to-all whole-genome alignment graph that lets us explore sequence conservation and variation, infer phylogeny, and identify recombination events. PGGB has been extensively evaluated (Andreace *et al.* 2023, Garrison *et al.* 2024) and applied to build the first draft human pangenome reference (Liao *et al.* 2023). However, PGGB is implemented in bash, which (a) makes it difficult to deploy on High-Performance

Received: 14 May 2024; **Revised:** 16 September 2024; **Editorial Decision:** 7 October 2024; **Accepted:** 10 October 2024

Computing (HPC) systems, (b) does not allow for a fine granular tuning of computing resources for different steps of the pipeline (Sztuka *et al.* 2024), and (c) limits its cluster scalability to one node. These limitations greatly hinder the broad application of large-scale pangenomes.

To compensate for that, we wrote *nf-core/pangenome*, a reference-unbiased approach to construct pangenome graphs. Mirroring PGGB, nf-core/pangenome is implemented in Nextflow (Di Tommaso *et al.* 2017). In contrast to PGGB, nf-core/pangenome can distribute the quadratic all-to-all base-level alignments across nodes of a cluster by splitting the approximate alignments into problems of equal size. We benchmarked the time spent on base-pair level alignments and show that it is reduced linearly with an increase in alignment problem chunks (Supplementary Material 5.5). We showcase the workflow's scalability by applying it to 1000 chromosome 19 human haplotypes and 2146 *Escherichia coli* sequences, which were built in less than half the time PGGB required while not increasing the CO2 equivalent (CO2e) emissions (Lannelongue *et al.* 2021).

2 Materials and methods

2.1 Pipeline overview

The pipeline's (Fig. 1a) input is a FASTA file compressed with *bgzip* (Li *et al.* 2009) containing the sequences to create the graph. Sequence names should follow the Pangenome Sequence Naming specification (PanSN-spec) (https://github.com/pangenome/PanSN-spec, last accessed October 2024). The primary output is a pangenome variation graph (Garrison *et al.* 2018) in the Graphical Fragment Assembly (GFA) format version 1 (http://gfa-spec.github.io/GFA-spec/GFA1.html, last accessed October 2024).

2.1.1 Core workflow

The core workflow of nf-core/pangenome mirrors PGGB (Fig. 1a) with additional enhancements: (a) All concurrent processes can be run in parallel. (b) Each process can be given individual computing resources.

The pipeline begins with an all-to-all alignment of the input sequences using the whole-chromosome pairwise sequence aligner WFMASH (https://github.com/waveygang/wfmash, last accessed October 2024), avoiding reference, order, or orientation bias, allowing every sequence to serve as a reference. In the pangenome graph induction step SEQWISH (Garrison and Guarracino 2023), an alignment to variation graph inducer, converts the sequence alignments into a variation graph. The graph is then simplified using SMOOTHXG (Garrison *et al.* 2024): A 1-dimensional (1D) graph embedding (Heumos *et al.* 2024) orders the graph's nodes to best match the nucleotide distances of the genomic paths of the graph. Next, the graph is split into partially overlapping segments. The sequences of each segment are realigned with a local Multiple Sequence Alignment (MSA) kernel, partial order alignment (POA) (Lee *et al.* 2002). Afterwards, the segments are laced back together into a variation graph. By default, the SMOOTHXG process is applied 3 times in order to smooth the edge effects at the boundaries of the segments. Finally, we employ GFAFFIX (Liao *et al.* 2023) to systematically condense redundant nodes within the graph.

Graph quality is assessed with ODGI (Guarracino *et al.* 2022), which provides statistics and visualizations. Optionally, variants can be called against any (reference) path(s) in the graph using *vg deconstruct* (Garrison *et al.* 2018). Results are summarized in a MultiQC (Ewels *et al.* 2016) report. Pipeline implementation details are given in Supplementary Material 5.1.

If desired, the pipeline performs community detection to identify clusters of related sequences in the pangenome graph, revealing biological patterns such as conserved or divergent regions across genomes (Supplementary Material 5.2), with the core workflow executed for each community in parallel.

3 Results

3.1 Building a 1000 haplotypes chr19 pangenome graph

The Human Pangenome Resource Consortium (HPRC) recently built a draft human pangenome of 90 haplotypes. However, haplotype data for thousands of individuals was already generated by the 1000 Genomes Project (1KGP) (The 1000 Genomes Project Consortium 2010). As a use case, we used nf-core/pangenome to build a graph of 1000 chromosome 19 haplotypes (Kuhnle *et al.* 2020) in 3 days, emitting 51.07 kg CO2e. PGGB took 7 days for the same task (56.32 kg CO2e). In Fig. 1b the pangenome growth curve generated with PANACUS (Liao *et al.* 2023) shows nucleotide growth as more haplotypes are added. The softcore pangenome, defined as sequences traversed by 95% of haplotypes, comprises the majority of the pangenome even with 1000 haplotypes. This stability may be due to the exclusion of complex regions like the centromere in the short-read data.

3.2 Building a 2146 sequences *E. coli* pangenome graph

To evaluate the pipeline's scalability, we built a pangenome graph of 2146 *E. coli* sequences. The nf-core/pangenome graph was completed in 10 days, emitting 175.18 kg CO2e, while PGGB could not finish within 30 days due to cluster time restrictions. For the growth curve (Fig. 1c) we excluded 130 plasmid sequences. The softcore pangenome remains stable at ~3Mb, but its size constitutes less than 10% of the total pangenome. This substantial pangenomic growth is likely driven by horizontal gene transfer, as bacteria incorporate genes from one another at various genomic locations. Other reasons could be sequencing errors or human contamination (Breitwieser *et al.* 2019).

4 Discussion

We implemented nf-core/pangenome, an easy-to-install, portable, and cluster-scalable pipeline for unbiased pangenome variation graph construction. It is the first pangenomic pipeline within the nf-core framework that enables the comparative analysis of gigabase-scale pangenome datasets. While tools like Minigraph (Li *et al.* 2020) or PGR-TK (Chin *et al.* 2023) also address pangenome analysis, nf-core/pangenome uniquely integrates this capability into the standardized nf-core framework, offering compatibility with a wide range of modular workflows and community-developed best practices.

The pipeline's core workflow has been successfully applied to *Neisseria meningitidis* (Yang *et al.* 2023), wild grapes (Cochetel *et al.* 2023), humans (Guarracino *et al.* 2023, Liao *et al.* 2023), grapevines (Guo *et al.* 2024), taurines (Milia *et al.* 2024), and rats (Villani *et al.* 2024) underpinning the

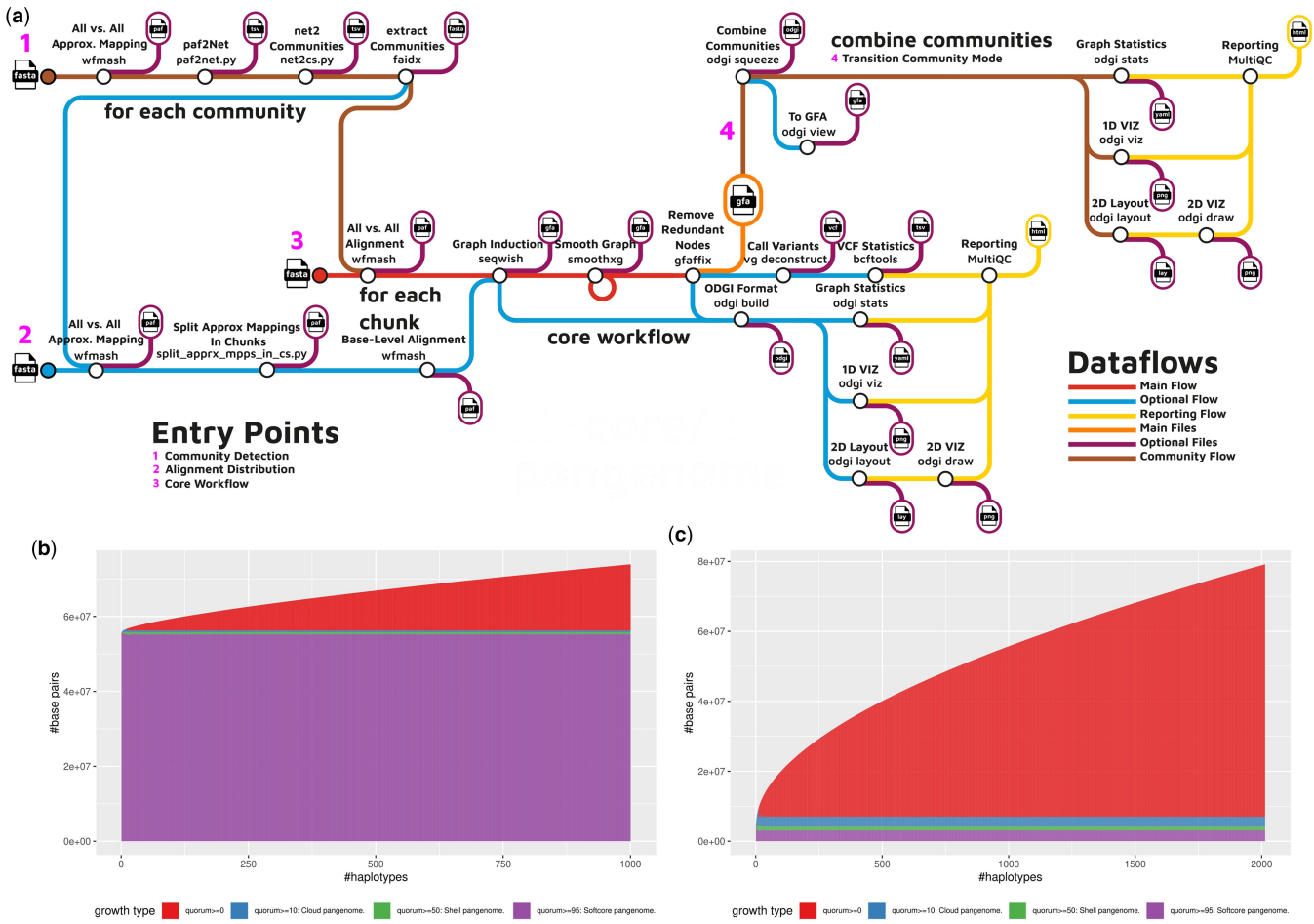

Figure 1. (a) Schematic representation of the nf-core/pangenome workflow processes and detailed analysis steps. The input consists of one FASTA file containing all sequences. The pipeline comes with three major entry points: (1) community detection, which identifies clusters of related sequences or regions in the pangenome graph to reveal biologically significant patterns like conserved or divergent areas across genomes (Supplementary Material 5.2), (2) alignment distribution, and (3) core workflow. Optional community detection (1) is performed on the input sequences. If selected, the heavy all-to-all base-pair level alignments (2) can be split into problems of equal size. nf-core/pangenome's core workflow (3) is a direct mirror of PGGB. If running in community mode, all communal graphs are combined into one (4) and the subsequent quality control subworkflow is executed. The output is a pangenome graph in GFA format. (b, c) Pangenome growth curves of the built pangenome graphs. Growth type is defined as the minimum fraction of haplotypes that must share a graph feature after each time a haplotype is added to the growth histograph. $quorum >= 0$: all sequences without any filtering are considered. $quorum >= 10$: sequences traversed by at least 10% of the haplotypes. $quorum >= 50$: sequences traversed by at least 50% of haplotypes. $quorum >= 95$: sequences traversed by 95% of haplotypes. (b) Pangenome growth curve of the chromosome 19 pangenome graph of 1000 haplotypes. (c) Pangenome growth curve of the *Escherichia coli* pangenome graph of 2013 haplotypes.

community effort to focus on a best-practice workflow to create reference-unbiased and sequence complete pangenome graphs. The modular domain-specific language (DSL) 2 pipeline structure facilitates easy exchange of processes with alternative tools, expanding its functionality and integration with other (sub-)workflows.

We have shown that we are able to perform all-vs-all base pair level alignments of thousands of sequences. When executed on an HPC, nf-core/pangenome's parallel workflow accelerates graph construction compared to PGGB. PGGB's inability to assign individual computational resources to each pipeline step leads to the allocation of one whole node of an HPC, despite the fact that some processes can only make use of one thread. This blocks valuable CPU cycles. In contrast, nf-core/pangenome leverages Nextflow's process management for optimal resource allocation, crucial for cloud-based executions.

Competing pipelines either lack workflow management system (Chin *et al.* 2023), or their workflow language of choice is e.g. Toil (Vivian *et al.* 2017, Hickey *et al.* 2024) which makes them less user-friendly, less cluster-efficient, and less portable (Wratten *et al.* 2021). nf-core/pangenome is currently the only pangenomics pipeline that is optionally monitoring its CO2 footprint. The measurements have shown that constructing extensive pangenome graphs, such as the 2146 *E. coli* graph, requires a considerable amount of energy. Therefore, we recommend assessing the rationale and methodology before conducting energy-intensive experiments.

Although we expect our pipeline to scale for future challenges, such as for the next HPRC phase which targets 350 individuals, further optimizations are possible: The IMplicit Pangenome Graph (IMPG) (https://github.com/ekg/impg, last accessed October 2024) tool extracts homologous loci from genomes mapped to a specific target region. This would allow us to break the whole genome multiple alignments into smaller pieces, construct a pangenome graph for each piece, and lace these together into a full graph with gfalace (https://github.com/pangenome/gfalace, last accessed October 2024).

We anticipate the pipeline, or its parts, will enhance current single linear reference analysis methods to explore whole

population variation instead of focusing on one reference only. Looking ahead, pangenome construction pipelines like nf-core/pangenome will play a pivotal role in studying entire populations, single-cell whole genome sequencing analysis, and constructing personalized (medical) pangenome references (Sirén *et al.* 2024).

Acknowledgements

We thank Matthias Seybold from QBiC for maintaining the Core Facility Cluster. We thank Sabrina Krakau from QBiC for giving feedback to the nf-co2footprint plugin section. We are grateful to the nf-core community for their support during the implementation of the pipeline. From the nf-core community, we want to thank Matthias Hörtenhuber, Maxime Garcia, Susanne Jodoin, Júlia Mir Pedrol, Adam Talbot, and Gisela Gabernet.

Supplementary data

Supplementary data are available at *Bioinformatics* online.

Conflict of interest

Author L.H. is employed by LaminLabs.

Funding

S.H. acknowledges funding from the Central Innovation Programme (ZIM) for SMEs of the Federal Ministry for Economic Affairs and Energy of Germany. This work was supported by the BMBF-funded de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI) (031A532B, 031A533A, 031A533B, 031A534A, 031A535A, 031A537A, 031A537B, 031A537C, 031A537D, and 031A538A). A.G. acknowledges support from the Human Technopole. S.N. acknowledges support from iFIT funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—EXC 2180—390900677 and CMFI under EXC 2124–390838134. We gratefully acknowledge support from NIH/NIDA U01DA047638 (E.G.), NIH/NIGMS R01GM123489 (E.G. and P.P.), and NSF PPoSS Award #2118709 (E.G. and P.P.), and the CITG (E.G.).

Data availability

Code and data resources for this manuscript and its figures are available in the public repository: https://github.com/subwaystation/pangenome-paper.

References

Andreace F, Lechat P, Dufresne Y *et al.* Comparing methods for constructing and representing human pangenome graphs. *Genome Biol* 2023;**24**:274.

Ballouz S, Dobin A, Gillis JA *et al.* Is it time to change the reference genome? *Genome Biol* 2019;**20**:159.

Breitwieser FP, Pertea M, Zimin AV *et al.* Human contamination in bacterial genomes has created thousands of spurious proteins. *Genome Res* 2019;**29**:954–60.

Chin C-S, Behera S, Khalak A *et al.* Multiscale analysis of pangenomes enables improved representation of genomic diversity for repetitive and clinically relevant genes. *Nat Methods* 2023;**20**:1213–21.

Cochetel N, Minio A, Guarracino A *et al.* A super-pangenome of the North American wild grape species. *Genome Biol* 2023;**24**:290.

Di Tommaso P, Chatzou M, Floden EW *et al.* Nextflow enables reproducible computational workflows. *Nat Biotechnol* 2017;**35**:316–9.

Eizenga JM, Novak AM, Sibbesen JA *et al.* Pangenome graphs. *Annu Rev Genomics Hum Genet* 2020;**21**:139–62.

Ewels P, Magnusson M, Lundin S *et al.* MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 2016;**32**:3047–8.

Garrison E, Guarracino A. Unbiased pangenome graphs. *Bioinformatics* 2023;**39**:btac743.

Garrison E, Sirén J, Novak AM *et al.* Variation graph toolkit improves read mapping by representing genetic variation in the reference. *Nat Biotechnol* 2018;**36**:875–9.

Garrison E, Guarracino A, Heumos S *et al.* Building pangenome graphs. *Nat Methods* 2024. https://doi.org/10.1038/s41592-024-02430-3

Guarracino A, Heumos S, Nahnsen S *et al.* ODGI: understanding pangenome graphs. *Bioinformatics* 2022;**38**:3319–26.

Guarracino A, Buonaiuto S, de Lima LG *et al.*; Human Pangenome Reference Consortium. Recombination between heterologous human acrocentric chromosomes. *Nature* 2023;**617**:335–43.

Guo L, Wang X, Ayhan DH *et al.* Super pangenome of grapevines empowers improvement of the oldest domesticated fruit. bioRxiv, 2024, preprint: not peer reviewed. https://doi.org/10.1101/2024.02.28.582440

Hein J. A new method that simultaneously aligns and reconstructs ancestral sequences for any number of homologous sequences, when the phylogeny is given. *Mol Biol Evol* 1989;**6**:649–68. https://doi.org/10.1093/oxfordjournals.molbev.a040577

Heumos S, Guarracino A, Schmelzle J-NM *et al.* Pangenome graph layout by path-guided stochastic gradient descent. *Bioinformatics* 2024;**40**:btae363.

Hickey G, Monlong J, Ebler J *et al.*; Human Pangenome Reference Consortium. Pangenome graph construction from genome alignments with minigraph-cactus. *Nat Biotechnol* 2024;**42**:663–73.

Kang M, Wu H, Liu H *et al.* The pan-genome and local adaptation of *Arabidopsis thaliana*. *Nat Commun* 2023;**14**:6259.

Kuhnle A, Mun T, Boucher C *et al.* Efficient construction of a complete index for pan-genomics read alignment. *J Comput Biol* 2020;**27**:500–13.

Lannelongue L, Grealey J, Inouye M *et al.* Green algorithms: quantifying the carbon footprint of computation. *Adv Sci* 2021;**8**:2100707.

Lee C, Grasso C, Sharlow MF *et al.* Multiple sequence alignment using partial order graphs. *Bioinformatics* 2002;**18**:452–64.

Leonard AS, Crysnanto D, Fang Z-H *et al.* Structural variant-based pangenome construction has low sensitivity to variability of haplotype-resolved bovine assemblies. *Nat Commun* 2022;**13**:3012.

Li H, Handsaker B, Wysoker A *et al.*; 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and samtools. *Bioinformatics* 2009;**25**:2078–9.

Li H, Feng X, Chu C *et al.* The design and construction of reference pangenome graphs with minigraph. *Genome Biol* 2020;**21**:265.

Liao W-W, Asri M, Ebler J *et al.* A draft human pangenome reference. *Nature* 2023;**617**:312–24.

Liu Y, Du H, Li P *et al.* Pan-genome of wild and cultivated soybeans. *Cell* 2020;**182**:162–76.e13.

Milia S, Leonard AS, Mapel XM *et al.* Taurine pangenome uncovers a segmental duplication upstream of KIT associated with depigmentation in white-headed cattle. bioRxiv, 2024, preprint: not peer reviewed. https://doi.org/10.1101/2024.02.02.578587

Minkin I, Pham S, Medvedev P *et al.* TwoPaCo: an efficient algorithm to build the compacted de Bruijn graph from many complete genomes. *Bioinformatics* 2017;**33**:4024–32.

Sayers EW, Beck J, Bolton EE *et al.* Database resources of the national center for biotechnology information. *Nucleic Acids Res* 2021;**49**:D10–7.

Sherman RM, Salzberg SL. Pan-genomics in the human genome era. *Nat Rev Genet* 2020;**21**:243–54.

Sirén J, Eskandar P, Ungaro MT *et al.* Personalized pangenome references. *Nat Methods* 2024. https://doi.org/10.1038/s41592-024-02407-2

Sztuka M, Kotlarz K, Mielczarek M *et al.* Nextflow vs. plain bash: different approaches to the parallelization of SNP calling from the whole genome sequence data. *NAR Genom Bioinform* 2024; **6**:lqae040.

The Computational Pan-Genomics Consortium. Computational pangenomics: status, promises and challenges. *Brief Bioinform* 2018; **19**:118–35.

The 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. *Nature* 2010;**467**:1061–73.

Traag VA, Waltman L, van Eck NJ *et al.* From Louvain to Leiden: guaranteeing well-connected communities. *Sci Rep* 2019;**9**:5233.

Villani F, Guarracino A, Ward RR *et al.* Pangenome reconstruction in rats enhances genotype-phenotype mapping and novel variant discovery. bioRxiv, 2024, preprint: not peer reviewed. https://doi.org/10.1101/2024.01.10.575041

Vivian J, Rao AA, Nothaft FA *et al.* Toil enables reproducible, open source, big biomedical data analyses. *Nat Biotechnol* 2017; **35**:314–6.

Weller CA, Andreev I, Chambers MJ *et al.*; NISC Comparative Sequencing Program. Highly complete long-read genomes reveal pangenomic variation underlying yeast phenotypic diversity. *Genome Res* 2023;**33**:729–40.

Wratten L, Wilm A, Göke J *et al.* Reproducible, scalable, and shareable analysis pipelines with bioinformatics workflow managers. *Nat Methods* 2021;**18**:1161–8.

Yang Z, Guarracino A, Biggs PJ *et al.* Pangenome graphs in infectious disease: a comprehensive genetic variation analysis of *Neisseria meningitidis* leveraging oxford nanopore long reads. *Front Genet* 2023;**14**:1225248.

Zhou Y, Yang L, Han X *et al.* Assembly of a pangenome for global cattle reveals missing sequences and novel structural variations, providing new insights into their diversity and evolutionary history. *Genome Res* 2022;**32**:1585–601.

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https://doi.org/10.1093/bioinformatics/btae609
Applications Note