stylo: a lightweight nf-core style nanopore assembly pipeline optimized for enteric bacteria

Arzoo Patel [1,2*], **Mohit Thakur** [1*], **Justin Kim** [1,2], **Peyton Smith** [1], **Lee S. Katz** [1], **Curtis Kapsak** [1,3], and **Jessica Chen** [1]

1 Enteric Diseases Laboratory Branch, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia **2** ASRT Inc., Contractor for National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. **3** Theiagen Genomics, Highlands Ranch, Colorado ***** These authors contributed equally.

Summary

Oxford Nanopore Technologies (ONT) sequencing is a promising technology with many potential applications in food safety. We have developed stylo, a lightweight nf-core style assembly workflow for ONT long-reads, specifically optimized for enteric bacteria. The pipeline downsamples, assembles, and performs post-processing and quality control by combining nanoq (E Steinig & L Coin, 2022), Rasusa (M B Hall, 2022), Flye (M Kolmogorov et al., 2019), Dnaapler (Bouras et al., 2024), Medaka ("Medaka," 2024), and BUSCO (M Seppey et al., 2019). All of stylo's dependencies are containerized and the pipeline is available on GitHub.

Statement of Need

There is a continuous need for foodborne outbreak detection in public health. To determine the scope or severity of a foodborne outbreak, short-read whole genome sequencing has often been used to generate isolate assemblies of enteric bacteria often which supports rapid and accurate outbreak detection (E M Ribot & K B Hise, 2016) (R E Timme et al., 2017). However, as nanopore long-read sequencing becomes more cost-effective and accurate, the need increases for streamlined assembly pipelines to support high-throughput surveillance processing of ONT sequenced isolates (N D Sanderson et al., 2024) (H H Mostafa, 2024). With the increased adoption of modern high-performance computing and cloud servers, pipelines built to leverage containerization and custom configurations allow for easy deployment on those servers. To address these needs, we have created stylo, a lightweight nf-core style nanopore assembly pipeline optimized for enteric bacteria (P Di Tommaso et al., 2017) (P A Ewels et al., 2020).

Stylo is developed for PulseNet, a molecular surveillance network for foodborne infections in the United States (P Gerner-Smidt et al., 2006). PulseNet facilitates the rapid detection of illness clusters and reduces the likelihood of outbreaks becoming large and widespread (B Tolar et al., 2019). Stylo utilizes a lookup table of PulseNet organism genome sizes, allowing users to run large and diverse datasets of enteric bacteria. There exists generalized nanopore assembly workflows such as Donut Falls (E Young & K Florek, 2025), whereas stylo is a streamlined workflow specialized for PulseNet to facilitate downstream genotyping.

Workflow Overview

1. Input: stylo requires a comma separated value file with columns for sample, fastq, genus, and species. Fastq files must comprise of long-reads generated on an ONT instrument.

40 Genus and species are used to automatically determine genome size via a lookup table
41 built into the pipeline.

2. Filtering and Downsampling: The pipeline filters out reads that are less than a user
    provided minimum length using nanoq. The resulting fastq is then subsampled to a user
    provided coverage via Rasusa.

3. Assembly: Flye is run on the subsampled fastq using the "–nano-hq" mode by default,
    expecting high-quality ONT reads. This parameter can be changed by the user.

4. Post-processing and Quality Control: The pipeline uses "dnaapler all" to reorient contigs
    to begin at a specific genes. The pipeline then uses Medaka to correct assembly sequences.
    Finally, the assembly quality is assessed via BUSCO, run with parameter mode set to
    "genome".

5. Output: The pipeline outputs files for each step. Some key files are the assembly by Flye,
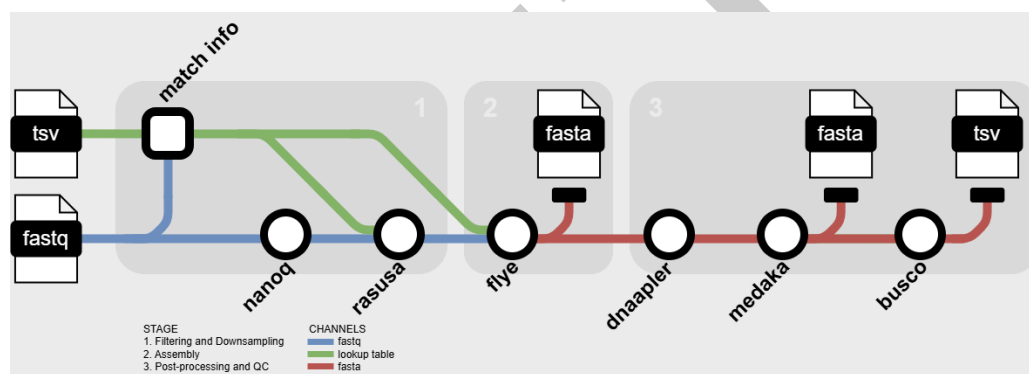   the final corrected assembly by Medaka, and the quality control summary by BUSCO.

Figure 1: Diagram of stylo steps.

Availability

Stylo is freely available and open-source. It can be downloaded from the GitHub repository
available at https://github.com/ncezid-biome/stylo.

Acknowledgements

These authors contributed equally as co-first: Arzoo Patel and Mohit Thakur. We acknowledge
helpful discussions from Joe Wirth.

References

B Tolar, L A Joseph, M N Schroeder, S Stroika, E M Ribot, K B Hise, & P Gerner-Smidt.
  (2019). An overview of PulseNet USA databases. *Foodborne Pathogens and Disease*,
  16(7), 457–462. https://doi.org/10.1089/fpd.2019.2637

Bouras, G., Grigson, S. R., Papudeshi, B., Mallawaarachchi, V., & Roach, M. J. (2024).
  Dnaapler: A tool to reorient circular microbial genomes. *Journal of Open Source Software*,
  9(93), 5968. https://doi.org/10.21105/joss.05968

E M Ribot, & K B Hise. (2016). Future challenges for tracking foodborne diseases. *EMBO
  Rep.*, *17*(11), 1499–1505. https://doi.org/10.15252/embr.201643128

E Steinig, & L Coin. (2022). Nanoq: Ultra-fast quality control for nanopore reads. *Journal of Open Source Software*, *7*(69), 2991. https://doi.org/10.21105/joss.02991

E Young, & K Florek. (2025). Donut falls. In *GitHub repository*. GitHub. https://github.com/UPHL-BioNGS/Donut_Falls

H H Mostafa. (2024). An evolution of nanopore next-generation sequencing technology: Implications for medical microbiology and public health. *Journal of Clinical Microbiology*, *62*(5), e00246–24. https://doi.org/10.1128/jcm.00246-24

M B Hall. (2022). Rasusa: Randomly subsample sequencing reads to a specified coverage. *Journal of Open Source Software*, *7*(69), 3941. https://doi.org/10.21105/joss.03941

M Kolmogorov, J Yuan, Y Lin, & P A Pevzner. (2019). Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology*, *37*, 540–546. https://doi.org/10.1038/s41587-019-0072-8

M Seppey, M Manni, & E M Zdobnov. (2019). BUSCO: Assessing genome assembly and annotation completeness. In M. Kollmar (Ed.), *Gene prediction: Methods and protocols* (pp. 227–245). Springer New York. https://doi.org/10.1007/978-1-4939-9173-0_14

Medaka. (2024). In *GitHub repository*. GitHub. https://github.com/nanoporetech/medaka

N D Sanderson, K M V Hopkins, M Colpus, M Parker, S Lipworth, D Crook, & N Stoesser. (2024). Evaluation of the accuracy of bacterial genome reconstruction with oxford nanopore R10.4.1 long-read-only sequencing [Journal Article]. *Microbial Genomics*, *10*(5). https://doi.org/10.1099/mgen.0.001246

P A Ewels, A Peltzer, S Fillinger, H Patel, J Alneberg, A Wilm, M U Garcia, P Di Tommaso, & S Nahnsen. (2020). The nf-core framework for community-curated bioinformatics pipelines. *Nature Biotechnology*, *38*, 276–278. https://doi.org/10.1038/s41587-020-0439-x

P Di Tommaso, M Chatzou, E W Floden, P P Barja, E Palumbo, & C Notredame. (2017). Nextflow enables reproducible computational workflows. *Nature Biotechnology*, *35*, 316–319. https://doi.org/10.1038/nbt.3820

P Gerner-Smidt, K Hise, J Kincaid, S Hunter, S Rolando, E Hyytiä-Trees, E M Ribot, & B Swaminathan. (2006). PulseNet USA: A five-year update. *Foodborne Pathogens and Disease*, *3*(1), 9–19. https://doi.org/10.1089/fpd.2006.3.9

R E Timme, H Rand, M Shumway, E K Trees, M Simmons, R Agarwala, S Davis, G E Tillman, S Defibaugh-Chavez, H A Carleton, W A Klimke, & L S Katz. (2017). Benchmark datasets for phylogenomic pipeline validation, applications for foodborne pathogen surveillance. *PeerJ*, *5*(e3893), e3893. https://doi.org/10.7717/peerj.3893