

Illumina and Nanopore Combo Assembly and Annotation (Eukaryotic)

Porechop¹ was used to trim residual adapter sequence from the Oxford Nanopore Technology (ONT) reads that may have been missed during basecalling and demultiplexing. *De novo* genome assemblies were generated from the Oxford Nanopore Technologies (ONT) read data with Flye² under the nano-hq (ONT high-quality reads) model. Additional Flye options initiate the assembly by first using reads longer than an estimated N50 based on a genome size of 12Mbp. Subsequent polishing used the Illumina read data with Pilon³ under default parameters. To reduce erroneous assembly artifacts caused by low quality nanopore reads, long read contigs with an average short read coverage of 15x or less were removed from the assembly.

Assembled genome sequences were annotated with funannotate⁴. Queried funannotate databases include: dbCAN, go, interpro, merops, mibig, pfam, repeats, and uniprot. Final assembly statistics were recorded with QUAST⁵ and are reported in the “*Assembly Metrics.tsv*” file. The assembly and annotation files are “*SampleName.fasta*”, “*SampleName.gff*”, and “*SampleName.gbk*” respectively. A summary of the assembled contigs can be found in each sample’s respective ‘*Contig Summary.tsv*’ file.

Tool	Version	Parameters
porechop	0.2.4	default parameters
flye	2.9.2	--asm-coverage 50 --genome-size 12000000 --nano-hq
pilon	1.24	default parameters
funannotate	1.8.15	interproscan and eggno3 mapper enabled
quast	5.2.0	default parameters

¹ An open source software for the QC and adapter trimming of ONT technologies.

<https://github.com/rrwick/Porechop>

² Mikhail Kolmogorov, Jeffrey Yuan, Yu Lin and Pavel Pevzner. Assembly of Long Error-Prone Reads Using Repeat Graphs. *Nature Biotechnology* (2019)

³ Walker, Bruce J et al. “Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement.” *PloS one* vol. 9,11 e112963. 19 Nov. 2014, doi:10.1371/journal.pone.0112963

⁴ Palmer J (2017) Funannotate: Fungal genome annotation scripts.

<https://github.com/nextgenusfs/funannotate>

⁵ Alexey Gurevich, Vladislav Saveliev, Nikolay Vyahhi and Glenn Tesler, QUAST: quality assessment tool for genome assemblies, *Bioinformatics* (2013) 29 (8): 1072-1075.