metagWGS: a workflow to analyse short and long HiFi metagenomic reads

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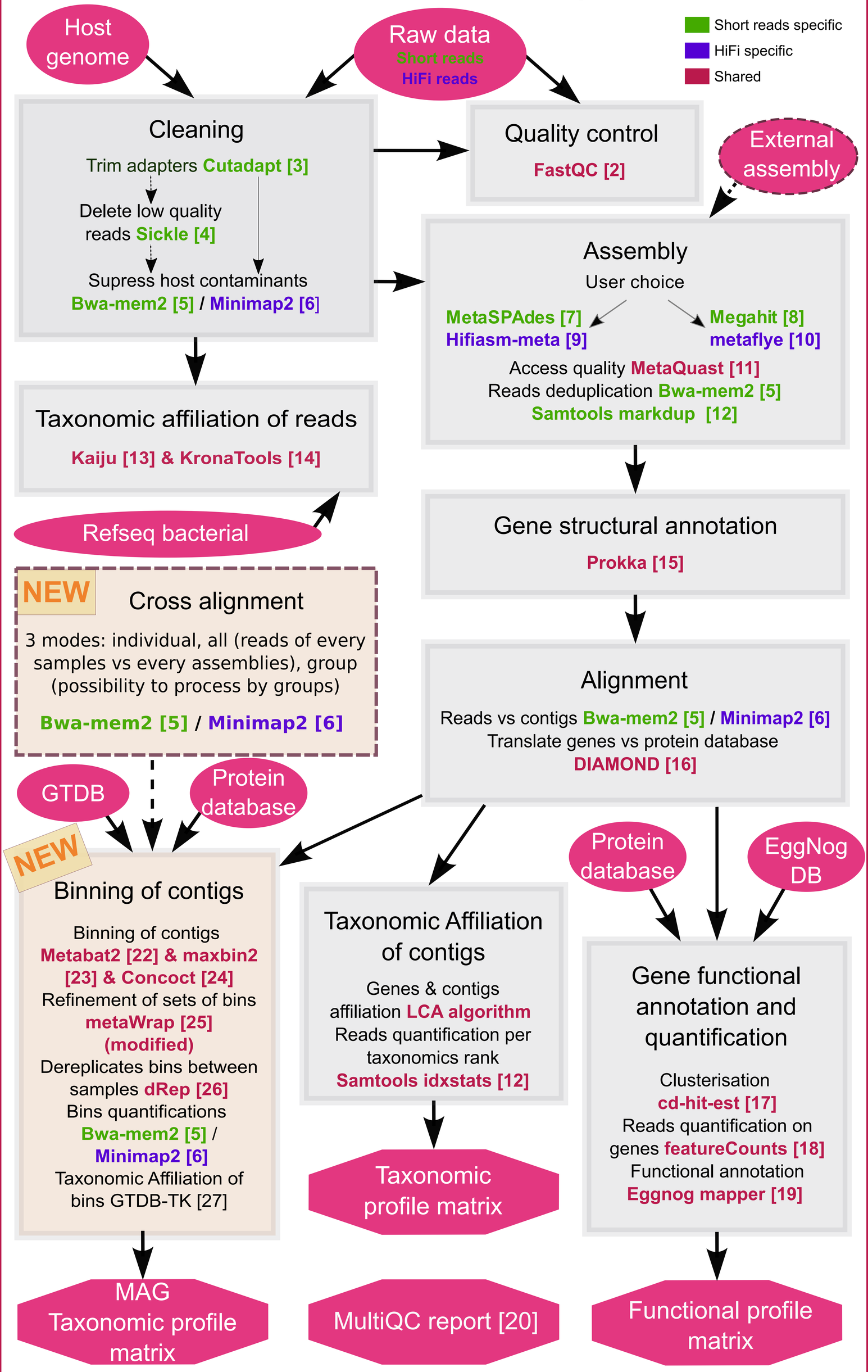
Production of whole metagenome assembly, functional and taxonomic profile

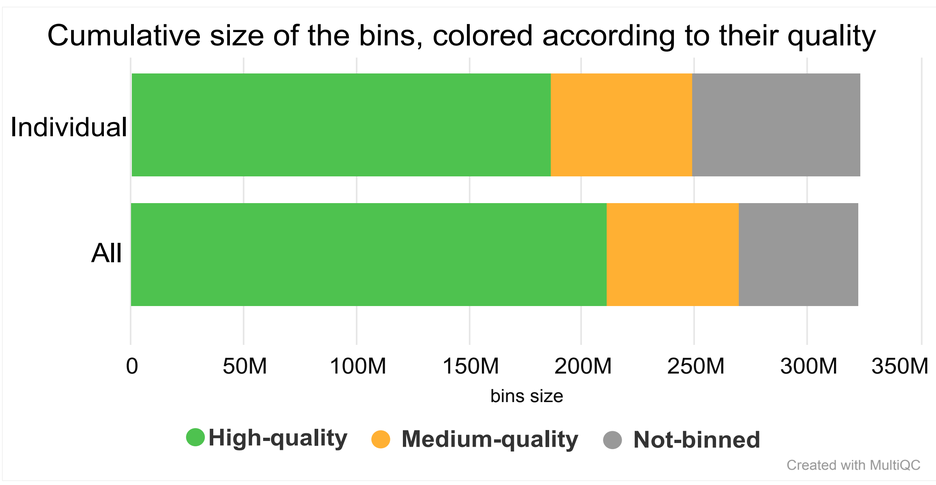


Workflow features

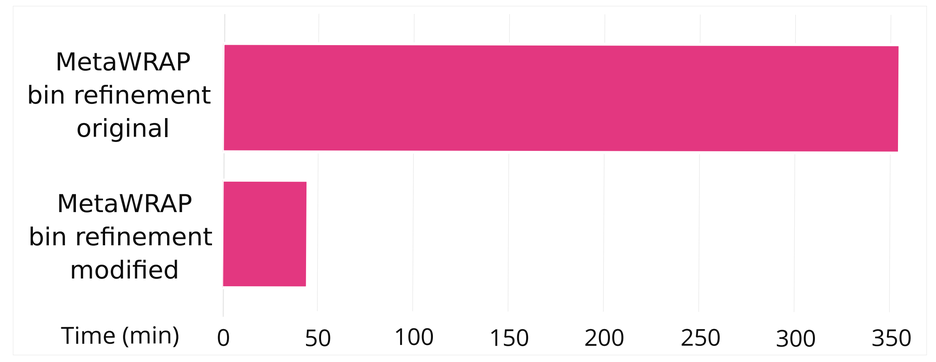
Type of NGS data:
whole genome shotgun sequencing (Illumina HiSeq3000 or NovaSeq, paired, 2*150bp ; PacBio HiFi reads, single-end)

Workflow:
a scalable and reproducible metagenomic analysis with a nextflow [1] pipeline using singularity [21] containers

Fully documented
https://forgemia.inra.fr/genotoul-bioinfo/metagwgs



The strategy of aligning reads of every samples (Cross-alignment: All) against each assembly improved the quality of binning. Tests were done on a synthetic mock composed of 142 bacteria and archea genomes with 3 samples of 66.651.100 Illumina paired-end reads (2x150bp).



Execution time in minutes of the original MetaWRAP bin refinement module [25] compare to the improved version implemented in metagGWS, on the synthetic mock. The improved version uses Checkm2 [28] instead of Checkm1 [29] and takes avantage of a custom resume parameter. The modified version gives very similar results.

Conclusion

The new version of **metagWGS (2.3)** allows the **analysis of Illumina short reads or PacBio HiFi long-reads sequencing data** and brings as a **major new feature the binning of contigs.**

The workflow proposes to use the abundance information contained in nearby samples to **improve the binning by implementing cross-alignment per sample set.**

We have also improved the performance of the **bins refinement step by dividing the execution time by 7**.

References

[1] Di Tommaso, P. et al. Nextflow enables reproducible computational workflows. Nat Biotechnol 35, 316–319 (2017).
[2] Andrews, S. FastQC: A Quality Control Tool for High Throughput Sequence Data. (2010).
[3] Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12 (2011).
[4] Joshi, N. A. & Fass, J. N. Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (2011).
[5] Vasimuddin, M., Misra, S., Li, H. & Aluru, S. Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. in 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS) 314–324 (2019).
[6] Li, H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34, 3094–3100 (2018).
[7] Nurk, S., Meleshko, D., Korobeynikov, A. & Pevzner, P. A. metaSPAdes: a new versatile metagenomic assembler. Genome Res. 27, 824–834 (2017).
[8] Li, D., Liu, C.-M., Luo, R., Sadakane, K. & Lam, T.-W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31, 1674–1676 (2015).
[9] Feng, X., Cheng, H., Portik, D. & Li, H. Metagenome assembly of high-fidelity long reads with hifiasm-meta. Nat Methods 19, 671–674 (2022).
[10] Kolmogorov, M. et al. metaFlye: scalable long-read metagenome assembly using repeat graphs. Nat Methods 17, 1103–1110 (2020).
[11] Mikheenko, A., Saveliev, V. & Gurevich, A. MetaQUAST: evaluation of metagenome assemblies. Bioinformatics 32, 1088–1090 (2016).
[12] Li, H. et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).
[13] Menzel, P., Ng, K. L. & Krogh, A. Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nat Commun 7, 11257 (2016).
[14] Ondov, B. D., Bergman, N. H. & Phillippy, A. M. Interactive metagenomic visualization in a Web browser. BMC Bioinformatics 12, 385 (2011).
[15] Seemann, T. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069 (2014).
[16] Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using DIAMOND. Nat Methods 12, 59–60 (2015).
[17] Fu, L., Niu, B., Zhu, Z., Wu, S. & Li, W. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28, 3150–3152 (2012).
[18] Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30, 923–930 (2014).
[19] Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P. & Huerta-Cepas, J. eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. Mol Biol Evol 38, 5825–5829 (2021).
[20] Ewels, P., Magnusson, M., Lundin, S. & Käller, M. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32, 3047–3048 (2016).
[21] Kurtzer, G. M., Sochat, V. & Bauer, M. W. Singularity: Scientific containers for mobility of compute. PLoS One 12, e0177459 (2017).
[22] Kang, D. D. et al. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. PeerJ 7, e7359 (2019).
[23] Wu, Y.-W., Simmons, B. A. & Singer, S. W. MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. Bioinformatics 32, 605–607 (2016).
[24] Alneberg, J. et al. Binning metagenomic contigs by coverage and composition. Nat Methods 11, 1144–1146 (2014).
[25] Uritskiy, G. V., DiRuggiero, J. & Taylor, J. MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. Microbiome 6, 158 (2018).
[26] Olm, M. R., Brown, C. T., Brooks, B. & Banfield, J. F. dRep: a tool for fast and accurate genomic comparisons that enables improved genome recovery from metagenomes through de-replication. ISME J 11, 2864–2868 (2017).
[27] Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P. & Parks, D. H. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 36, 1925–1927 (2020).
[28] Chklovski, A., Parks, D. H., Woodcroft, B. J. & Tyson, G. W. CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. Nat Methods 20, 1203–1212 (2023).
[29] Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P. & Tyson, G. W. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25, 1043–1055 (2015).

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Perspectives

Better assembly of the minority species when the sequencing depth is not sufficient: implementation of co-assembly (by giving the possibility to normalize data first).

Improve the performance of the workflow: replacing Prokka [15] with other tools.

Long term perspectives: enable the annotation of antibiotic resistance genes and of the mobilome