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Baargin: a Nextflow workflow for the automatic analysis of bacterial genomics data with a focus on Antimicrobial Resistance

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Summary

The emergence and development of Antimicrobial Resistance (AMR) is a global health problem, that could cause about 10 million deaths yearly by 2050 ([Thompson, 2022](#)). The study of the genomes of these (multi)resistant bacterial strains is of high importance to understand emergence and circulation of the resistance. In the past couple of decades, high throughput sequencing technologies have seriously improved and it has become more affordable to sequence the full genomes of hundreds of bacterial strains at a time. As a counterpart, these experiments produce large amount of data that needs to be analysed by various bioinformatics methods and tools for reconstructing the genomes and therefore identify their specific features and the genetic determinants of the AMR. For automating the bioinformatics analysis of multiple strains, we have developed a Nextflow ([DI Tommaso et al., 2017](#)) workflow called *baargin* (Bacterial Assembly and Antimicrobial Resistance Genes detection In Nextflow) <https://github.com/jhayer/baargin>. It enables to conduct sequencing reads quality control, genome assembly and annotation, Multi-Locus Sequence Typing and plasmid identification, as well as antimicrobial resistance determinants detection, and pangenome analysis. The use of Nextflow, a workflow management system, makes our workflow portable, flexible, and able to conduct reproducible analyses.

Statement of need

High Throughput Sequencing technologies produce a significant amount of data and researchers are producing genomics data all over the world on a daily basis. These technologies are notably used for studying bacterial genomes in order to understand the spread of bacterial pathogens and their resistance to antibiotics. In the bacterial genomics field, it is possible to sequence the DNA from multiple bacterial strains at the same time. The analysis of these sequencing data requires the use of a wide range of bioinformatics programs to be able to identify the genes and their functions, and among those, the genes and mutations conferring resistance to antimicrobial drugs. In order to make the results of these analyses comparable, it is crucial to standardise, automate and parallelise all the steps. The *baargin* workflow allows the user to perform a complete *in silico* analysis of bacterial genomes, from the quality control of the raw data, to the detection of AMR genes and mutations, on multiple datasets of the same bacterial species in parallel. It compiles and summarise the results from all the analysis steps, allowing comparative studies. As a last step, *baargin* performs a pangenome analysis of all the strains provided, producing the basis for the construction of a phylogenetic tree. The use of Nextflow and containers ensures the reproducibility of the data analysis. Only few bacterial genomics

workflows are available, like Bactopia (Petit III, 2020), which is highly flexible and complete in term of tools available. Therefore, we needed a lighter workflow, with only a few tools and databases installed for our collaborators that have only limited computing resources and storage. Also, *baargin* is specifically designed for detecting AMR genes and plasmid features, and include a decontamination step of the assembly, allowing the downstream analyses to be performed especially on the contigs belonging to the targeted species.

Materials and Methods

Features

Baargin is designed to automatically parallelise workflow steps. It does not require manual intervention from the users between steps. Each workflow step, called process, uses containers, via Docker or Singularity, which also greatly improves traceability and reproducibility. Additional processes can be easily added in the future as the workflow is designed in modules, making it flexible for adding or removing steps.

Workflow

Figure 1 describes the workflow:

1. Input can be either a folder containing paired-end short reads (fastq format), a folder containing already assembled contigs (fasta format files), or an index file containing the paths to pair-end short reads (fastq files) and to long reads (ONT, fastq file) for the same sample/strain in order to perform hybrid assembly. If assembled contigs are provided, the analysis will start at step 4.
2. Quality check and adapters removal is performed on the short reads using Fastp (Chen et al., 2018).
3. De novo assembly is run using SPAdes (Prjibelski et al., 2020) if only short reads were provided, and with Unicycler (Wick et al., 2017) for a hybrid assembly when short and long reads are provided.
4. Taxonomic assignment of the contigs is performed using Kraken2 (Wood et al., 2019) and the contigs classified at the taxonomic level provided by the user (with the taxid, and including the children taxa) are retrieved and therefore named as “*deconta*” for decontaminated contigs (Lu et al., 2022). The dataset containing all the contigs are named as “*raw*”. From here all the steps except the annotation (8) will be performed on both sets of contigs “*raw*” and “*deconta*”.
5. A quality check of the assembly is conducted using Quast (Gurevich et al., 2013) and BUSCO (Manni et al., 2021). For BUSCO, the users have the possibility to specify the taxonomic lineage database to use for searching the housekeeping genes (at the class level of the strain to analyse for example: *enterobacterales_odb10*)
6. The contigs (*raw* and *deconta*) are then screened to identify the sequence type of the strain using the MLST tool (Multi-Locus Sequence Typing) (Seemann, 2022).
7. The contigs are subsequently submitted to plasmid identification using PlasmidFinder (Carattoli et al., 2014) and additionally with Platon if the user provides a database for it (Schwengers et al., 2020).
8. Antimicrobial Resistance Genes (ARGs) are then searched in the contigs using both CARD RGI (Alcock et al., 2023) and the NCBI AMRFinderPlus (Feldgarden et al., 2021). For certain species only, AMRFinderPlus can also detect some mutations conferring resistance, if the user provides that option.
9. A genome annotation is performed on the *deconta* contigs using Prokka by default (Seemann, 2014), or using Bakta (Schwengers et al., 2021) if the user provides a database for it.
10. Once all the strains datasets provided are annotated a pangenome analysis is done using Roary (Page et al., 2015).

Output

The results are located in a nested folder architecture. For each dataset, a folder with the sampleID is created, and contains 5 subfolders: - qc - assembly - AMR - annotation - plasmids. These subfolders contain the main outputs from the concerned analyses. Additionally, at the root of the results folder (indicated by the user) 2 folders are created: *pangenome*, containing the results for Roary, and *compile_results* that contains summary files for each analysis where the results for all datasets provided in input are compiled as presence/absence matrices for further comparative analyses.

Discussion and conclusions

We presented here an easy-to-use workflow for Bacterial Assembly and Antimicrobial Resistance Genes detection In Nextflow: *baargin*. It allows the users to analyse genomic datasets from short and long sequencing reads, of several bacterial strains from the same species in one command line. The workflow will automatically assemble the genomes, check for contamination and specifically extract the sequences that belong to the expected taxon. It will then identify their sequence type and screen the assemblies for plasmids sequences and ARGs. The fact that *baargin* is implemented in Nextflow and is based on containers makes the analyses reproducible. Its modular design makes it easy to customise and extend, by adding new modules for new processes.

Figures

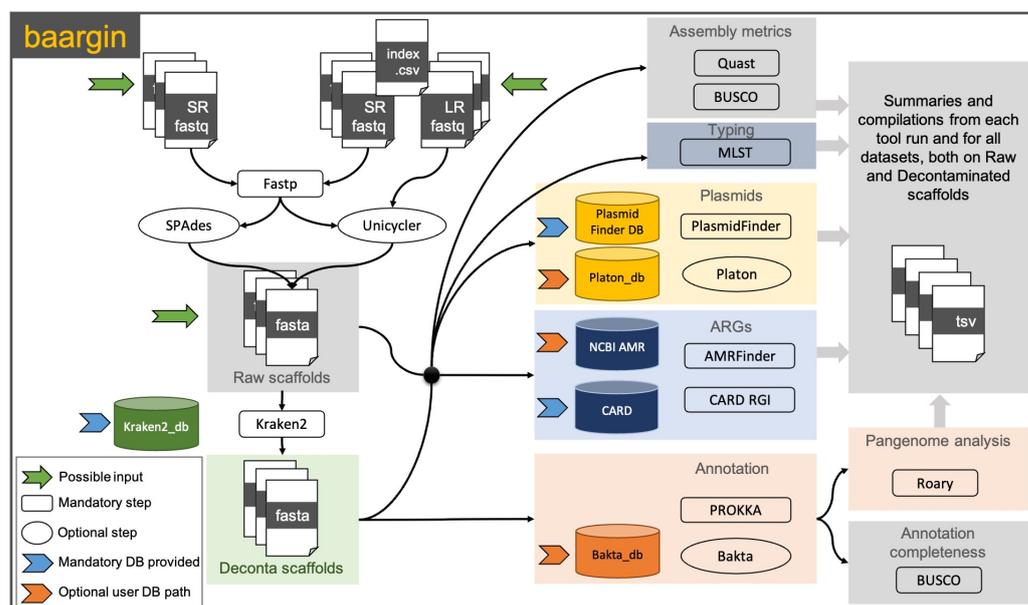


Figure 1: Flowchart of baargin workflow.

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paper. <https://bioinfo.ird.fr> <http://www.southgreen.fr>

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