GHRU (Genomic Surveillance of Antimicrobial Resistance) Retrospective 1 Bioinformatics Methods V.4

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ABSTRACT

A description of the pipelines used for analysing genome data from the retrospective 1 project of the NIHR Global Health Research Unit (Genomic Surveillance of Antimicrobial Resistance)

All genome sequence data were processed using versioned Nextflow [1] workflows and associated Docker [2] containers covering the foundational analyses of de novo assembly,mapping-based SNP phylogeny, MLST assignation and AMR determinant detection (table 1). The exact steps performed can be derived from examination of the pipeline code but each workflow will be described in brief.

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A description of the pipelines used for analysing genome data from the retrospective 1 project of the NIHR Global Health Research Unit (Genomic Surveillance of Antimicrobial Resistance)

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1 De novo assembly

reads trimming and adapter removal using trimmomatic (0.38) [3], read correction using lighter (1.1.1) [4], downsampling to 100x coverage using seqtk (1.3) [5], read merging using flash (1.2.11) [6], assembly using SPAdes (3.12.0) [7]. Quality control was performed using fastqc (0.11.8) [8], multiqc (1.7) [9] and qualifyr (1.4.4) [10]. Species identification was carried out by bactinspector (0.1.3) [11] and contamination checked using Confindr (0.7.2) [12].

2 Mapping based phylogeny

Reads were trimmed as described for de novo assembly and mapped to a reference with bwa mem (0.7.17) [13], variants called and filtered using bcf tools (1.9) [14] and the filtering conditions for a low quality position being

'%QUAL<25 || FORMAT/DP<10 || MAX(FORMAT/ADF)<2 || MAX(FORMAT/ADR)<2 || MAX(FORMAT/AD)/SUM(FORMAT/DP)<0.9 || MQ<30 || MQ0F>0.1'. A pseudoalignment where each sample has a base relative to the reference sequence. Missing bases and low quality bases are encoded using the - and N characters. The alignment was used to generate a maximum likelihood tree using iqtree (1.6.8) [15,16] and ultrafast bootstraps (parameters -m GTR+G -alrt 1000 -bb 1000).

3 AMR determinant detection

The ARIBA software (2.14.4) [17] was used to detect acquired genes using the NCBI database [18] (downloaded 2019-10-30) and the pointfinder database (downloaded 2019-12-11) adapted for Ariba [19].

4 MLST detection

The ARIBA software (2.14.4) [17] was used to determine the 7-locus MLST type using the profile and alleles found in the pubmlst database [20,21] (downloaded 2019-12-20).

5 Table 1: Nextflow workflows

Workflow name	Workflow link	Docker hub Container(s) used	Version at publication
De novo assembly	https://gitlab.com/cgps/ghru/pipelines/assembly	bioinformant/ghru-assembly:version OR registry.gitlab.com/cgps/ghru/pipelines/assembly:version	1.5.5
Mapping based SNP phylogeny	https://gitlab.com/cgps/ghru/pipelines/snp_phylogeny	bioinformant/ghru-snp-phylogeny:version OR registry.gitlab.com/cgps/ghru/pipelines/snp_phylogeny:version	1.2.2
AMR determinant detection	https://gitlab.com/cgps/ghru/pipelines/dsl2/pipelines/amr_prediction	bioinformant/ghru-amr-prediction:version OR registry.gitlab.com/cgps/ghru/pipelines/dsl2/pipelines/amr_prediction	1.0
MLST	https://gitlab.com/cgps/ghru/pipelines/dsl2/pipelines/mlst	bioinformant/ghru-mlst:version OR registry.gitlab.com/cgps/ghru/pipelines/dsl2/pipelines/mlst:version	1.0

6 References

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