

INPUT FASTQ Data

Base programs:
Nextflow C and C++
Python
Numpy

QC and Host Filtering

Remove Low-Quality Reads ($\underline{\text{Trimmomatic and }}$ $\underline{\text{Trimmomatic stats.py}}$)

Identify host reads ($\underline{\text{BWA}}$)

Remove host reads ($\underline{\text{Samtools and samtoolsidx stats.py}}$)

Convert file types ($\underline{\text{Bedtools}}$) $\underline{\text{Bedtools}}$ $\underline{\text{Remove Low-Quality Reads }}$ $\underline{\text{Remove BuildHostIndex AlignReads ToHost Remove HostDNA}}$ $\underline{\text{Remove HostDNA}}$

Resistome Analysis

Microbiome Analysis

Identify AMR reads (<u>BWA</u>)

- - → AlignToAMR

Apply gene fraction (ResistomeAnalyzer)

_ - → RunResistome

Build count matrix (amr_long_to_wide.py)

_ _ → ResistomeResults

Extract reads mapped to SNP genes (AWK)

'_ _ → ExtractSNP

Confirm SNPs required for resistance (RGI)

- - → RunRGI

Combine AMR counts (amr_long_to_wide.py)

_ _ → Confirmed ResistomeResults

Rarefy (<u>RarefactionAnalyzer</u>)

_ - → RunRarefaction

Identify SNPs (SNPFinder, Freebayes)

L _ → RunSNPFinder, RunFreebayes

OUTPUT resistome count matrix

Build kraken k-mer DB (download is already built)

Build k-mer index for each sample, match to DB (<u>kraken</u>)

- → RunKraken

Build count matrix (kraken_long_to_wide.py)

'_ _ → KrakenResults
FilteredKrakenResults

OUTPUT microbiome count matrix

<u>Underline indicates a program/script needed to run Nextflow pipeline</u> Italics indicates an output directory from Nextflow pipeline. Resistome analysis performs the same steps for de-duplicated alignments.