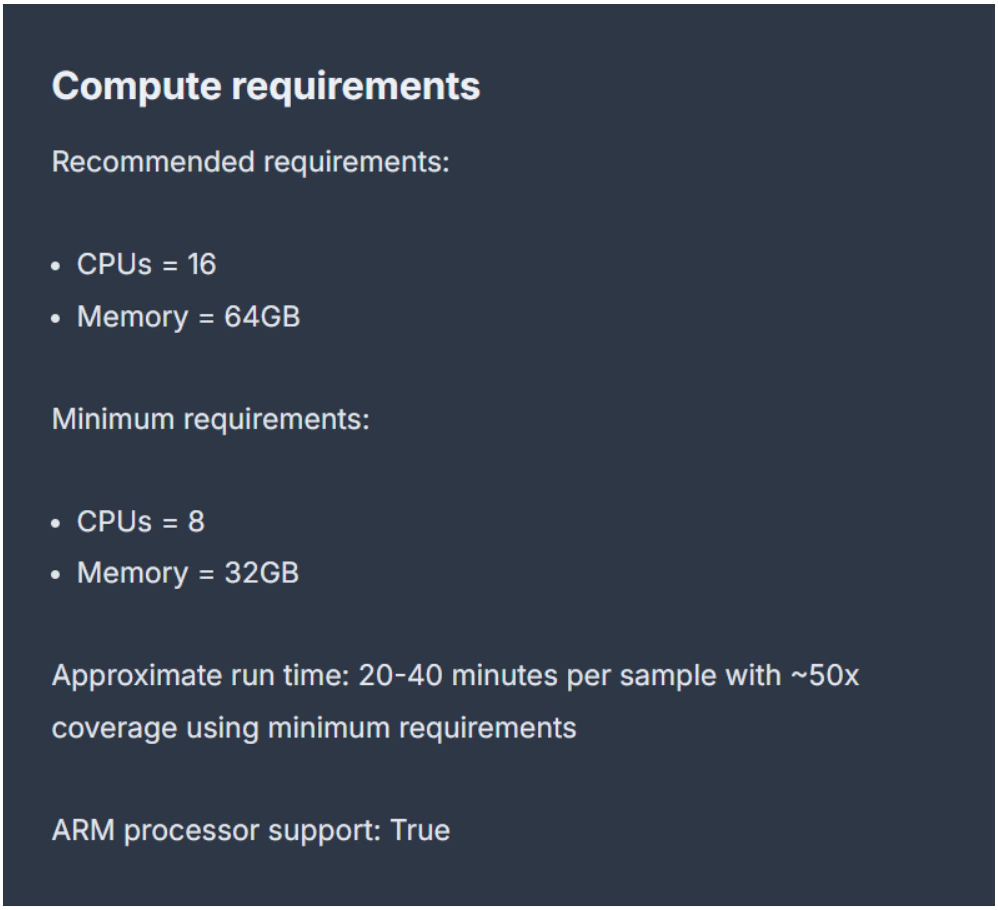
Install Nanopore EPI2ME software on Windows computer

Version: March 2025

Minimal Requirements for Computer/Laptop to install and run Epi2ME:



Compute requirements

Recommended requirements:

- CPUs = 16
- Memory = 64GB

Minimum requirements:

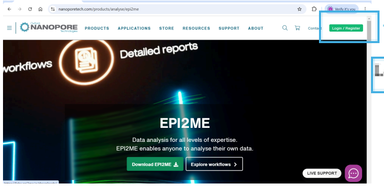
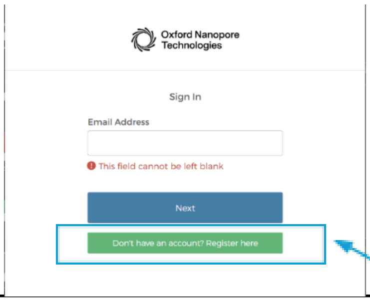
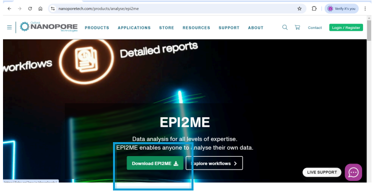
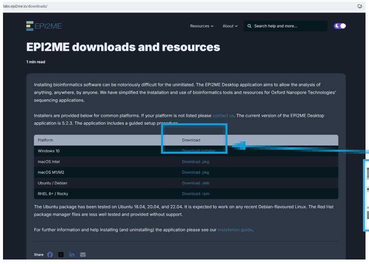
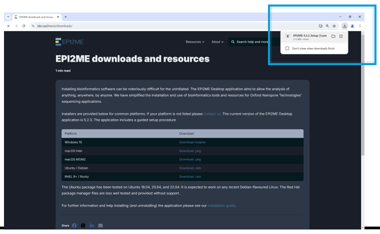
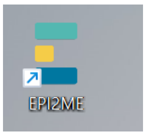
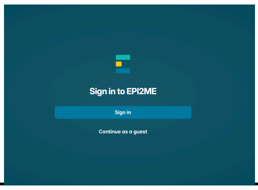
- CPUs = 8
- Memory = 32GB

Approximate run time: 20-40 minutes per sample with ~50x coverage using minimum requirements

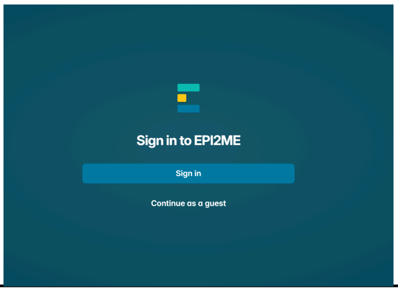
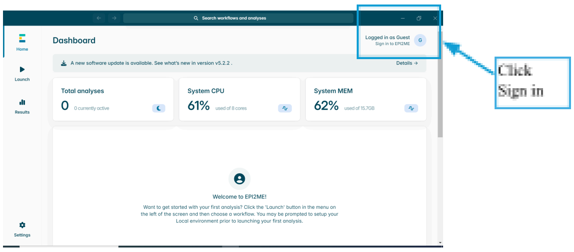
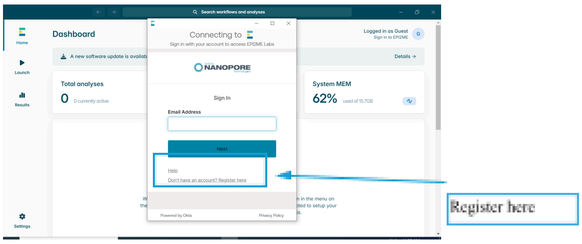
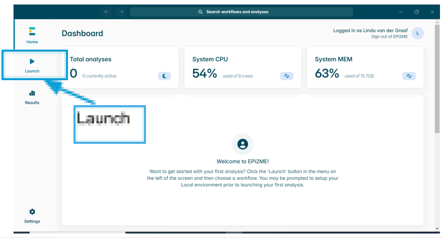
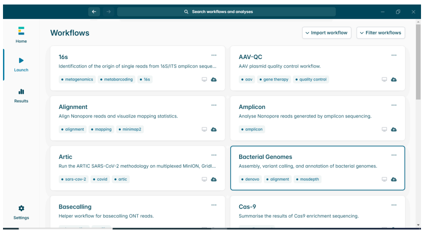
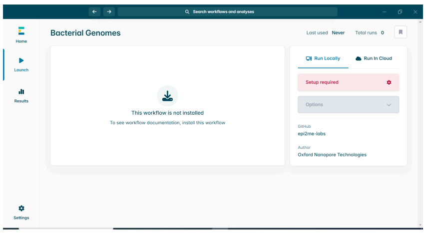
ARM processor support: True

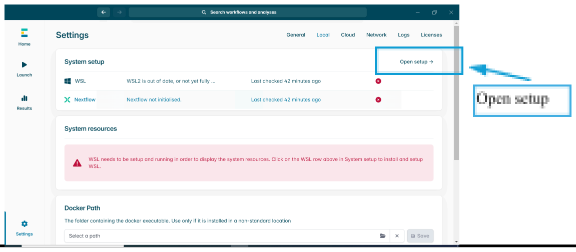
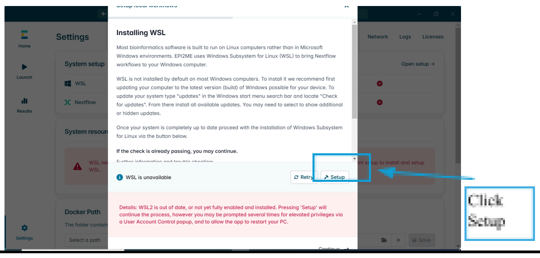
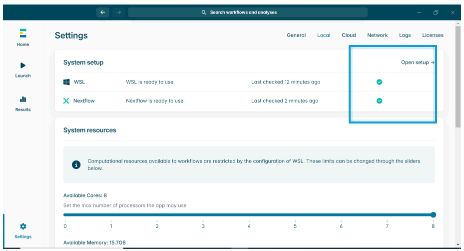
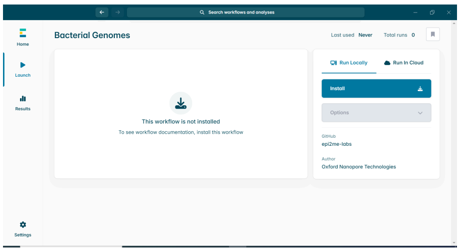
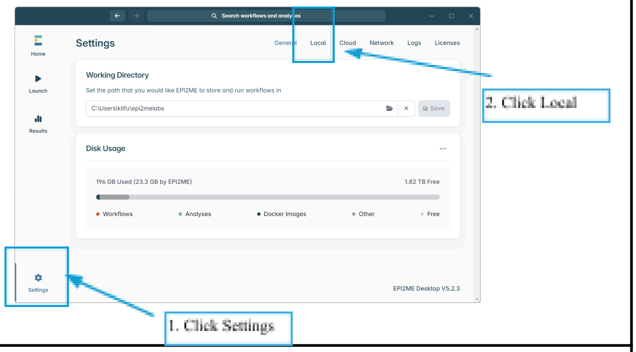
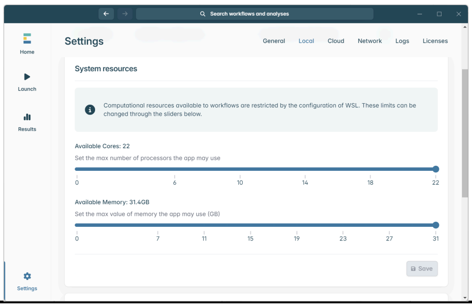
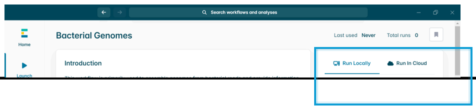
Install Nanopore Epi2Me software

Install Nanopore Epi2ME software on Windows computer

Navigate to Nanopore Epi2Me website: https://nanoporetech.com/products/analyse/epi2me Click on Login / Register	 Login here
Login with an existing account Or Create a new account by clicking “Don’t have an account? Register here” After registration, login with the new account	 Register here
Click on “Download EPI2ME”	 Start download
Click on “Windows 10” Platform – Download installer Additional: This page contains also the “installation guide” for help to (un)install the software	 Download Windows 10 installer
Wait till download has finished and click on the downloaded file to start installation of EPI2ME	 Click on downloaded file
After installation, the EPI2ME software is available and a desktop shortcut is created	
EPI2ME software can be started by clicking on Desktop Shotcut Icon	

Install Nanopore EPI2ME - Bacterial Assembly and Annotation Workflow

Open EPI2ME software and "Sign in" If you do not have an account, click "Continue as a guest"	
As guest: Click "Sign in to EPI2ME"	 A new software update is available. See what's new in version v5.2.2 . Welcome to EPI2ME! Want to get started with your first analysis? Click the 'Launch' button in the menu on the left of the screen and then choose a workflow. You may be prompted to setup your Local environment prior to launching your first analysis.
As guest: Click "Don't have an account? Register here" Register and login	 Sign in with your account to access EPI2ME Labs
After login, click "Launch"	 Welcome to EPI2ME! Want to get started with your first analysis? Click the 'Launch' button in the menu on the left of the screen and then choose a workflow. You may be prompted to setup your Local environment prior to launching your first analysis.
Click on "Bacterial Genomes"	 16s: Identification of the origin of single reads from 16S/ITS amplicon seque... AAV-QC: AAV plasmid quality control workflow. Alignment: Align Nanopore reads and visualize mapping statistics. Amplicon: Analyse Nanopore reads generated by amplicon sequencing. Artic: Run the ARTIC SARS-CoV-2 methodology on multiplexed MinION, Gridl... Bacterial Genomes: Assembly, variant calling, and annotation of bacterial genomes. Basecalling: Helper workflow for basecalling ONT reads. Cas-9: Summarise the results of Cas9 enrichment sequencing.
Normally, setup is required before installing the workflow Click on "Setup required"	 This workflow is not installed To see workflow documentation, install this workflow GitHub: epi2me-labs Author: Oxford Nanopore Technologies

Check if WSL and Nextflow are installed. If not (red cross), click “open setup”	 System setup WSL — WSL2 is out of date, or not yet fully ... — Last checked 42 minutes ago Nextflow — Nextflow not initialised. — Last checked 42 minutes ago System resources WSL needs to be setup and running in order to display the system resources. Click on the WSL row above in System setup to install and setup WSL. Docker Path The folder containing the docker executable. Use only if it is installed in a non-standard location Select a path
To install WSL, click “Setup” to start installation of WSL and Nextflow	 Installing WSL Most bioinformatics software is built to run on Linux computers rather than in Microsoft Windows environments. EPI2ME uses Windows Subsystem for Linux (WSL) to bring Nextflow workflows to your Windows computer. WSL is not installed by default on most Windows computers. To install it we recommend first updating your computer to the latest version (build) of Windows possible for your device. To update your system type "updates" in the Windows start menu search bar and locate "Check for updates". From there install all available updates. You may need to select to show additional or hidden updates. Once your system is completely up to date proceed with the installation of Windows Subsystem for Linux via the button below. **If the check is already passing, you may continue.** WSL is unavailable Details: WSL2 is out of date, or not yet fully enabled and installed. Pressing 'Setup' will continue the process, however you may be prompted several times for elevated privileges via a User Account Control popup, and to allow the app to restart your PC.
After installation of WSL and Nextflow, check if installation was successful – green checkmark will appear WSL and Nextflow are ready to use	 System setup WSL — WSL is ready to use. — Last checked 12 minutes ago Nextflow — Nextflow is ready to use. — Last checked 2 minutes ago System resources Computational resources available to workflows are restricted by the configuration of WSL. These limits can be changed through the sliders below. Available Cores: 8 Set the max number of processors the app may use Available Memory: 15.7GB
Go back to “Launch” Select “Bacterial Genomes” Click “Install” (in screen Run Locally) Please note: Installation can take very long (up to hours)	 Bacterial Genomes Last used Never Total runs 0 This workflow is not installed To see workflow documentation, install this workflow GitHub: epi2me-labs Author: Oxford Nanopore Technologies
Set system resources to maximum 1. Click “Settings” 2. Click “Local”	 Working Directory Set the path that you would like EPI2ME to store and run workflows in C:\Users\klifu\epi2melabs Disk Usage 196 GB Used (23.3 GB by EPI2ME) 1.82 TB Free EPI2ME Desktop V5.2.3
Slide both “Available cores” and “Available memory” to maximum	 System resources Computational resources available to workflows are restricted by the configuration of WSL. These limits can be changed through the sliders below. Available Cores: 22 Set the max number of processors the app may use Available Memory: 31.4GB Set the max value of memory the app may use (GB)
	 Bacterial Genomes Last used Never Total runs 0 Introduction

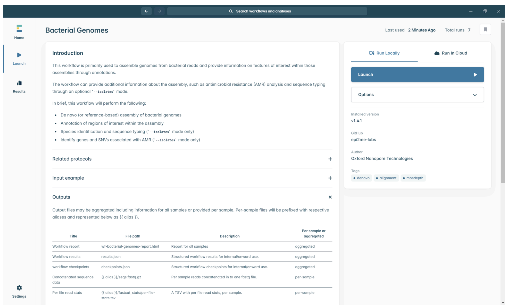
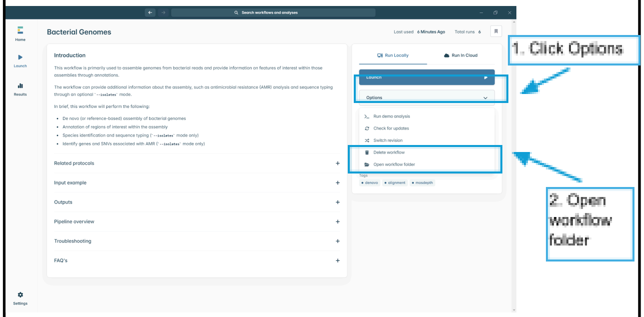
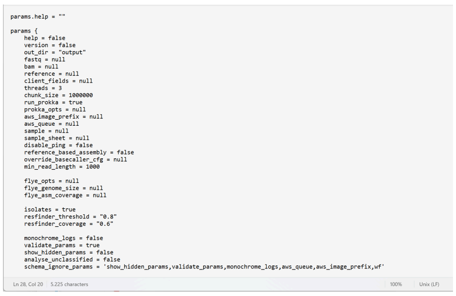
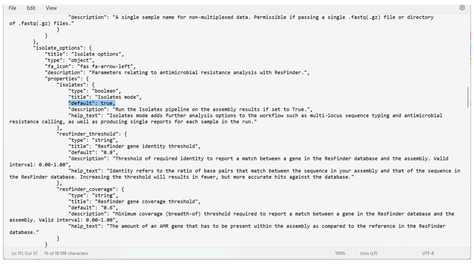
After successful installation of the Bacterial Genomes Workflow, the button "Launch" will appear, to be used to analyse Nanopore sequencing data.	

ADVANCED EPI2ME installation options – change Workflow files

Please note: Described modifications can only be performed by advanced users who know what they are doing

1. Advanced installation; change workflow to "isolates mode". Default setting is that isolates mode of bacterial genomes workflow it turned off. This isolates mode will add analysis like species identification, MLST STs and Resfinder result.

Follow the following steps to turn isolates mode on.

The isolates mode of the Bacterial Genomes workflow will add species identification, MLST STs and AMR gene analysis. Default setting is that this isolates mode is turned off	
Navigate to Bacterial Genomes Workflow 1. Click "Options" 2. Click "Open workflow folder"	
Open file "nextflow.config" in text editor Change: "isolates = false" in "isolates = true" Save file	
Open file "nextflow_schema.json" in text editor Change for isolates mode: ""default": false," in "default": true," Save file	

Bacterial Genomes

Last used 2 Minutes Ago Total runs 7

Introduction

This workflow is primarily used to assemble genomes from bacterial reads and provide information on features of interest within those assemblies through annotations.

The workflow can provide additional information about the assembly, such as antimicrobial resistance (AMR) analysis and sequence typing through an optional `--isolates` mode.

In brief, this workflow will perform the following:

- De novo (or reference-based) assembly of bacterial genomes
- Annotation of regions of interest within the assembly
- Species identification and sequence typing (`--isolates` mode only)
- Identify genes and SNVs associated with AMR (`--isolates` mode only)

Related protocols

Input example

Outputs

Output files may be aggregated including information for all samples or provided per sample. Per-sample files will be prefixed with respective aliases and represented below as {{ alias }}.

Title	File path	Description	Per sample or aggregated
Workflow report	wf-bacterial-genomes-report.html	Report for all samples	aggregated
Workflow results	results.json	Structured workflow results for internal/onward use.	aggregated
workflow checkpoints	checkpoints.json	Structured workflow checkpoints for internal/onward use.	aggregated
Concatenated sequence data	{{ alias }}/seqs.fastq.gz	Per sample reads concatenated in to one fastq file.	per-sample
Per file read stats	{{ alias }}/fastcat_stats/per-file-stats.tsv	A TSV with per file read stats, per sample.	per-sample

Installed version
v1.4.1

GitHub
epi2me-labs

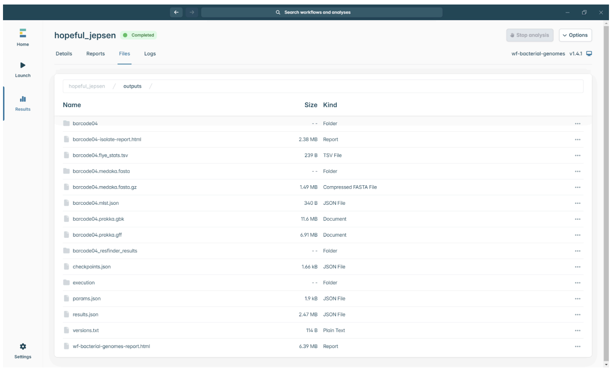
Author
Oxford Nanopore Technologies

Tags
denovo alignment mosdepth

```
params.help = ""

params {
    help = false
    version = false
    out_dir = "output"
    fastq = null
    bam = null
    reference = null
    client_fields = null
    threads = 3
    chunk_size = 1000000
    run_prokka = true
    prokka_opts = null
    aws_image_prefix = null
    aws_queue = null
    sample = null
    sample_sheet = null
    disable_ping = false
    reference_based_assembly = false
    override_basecaller_cfg = null
    min_read_length = 1000

    flye_opts = null
    flye_genome_size = null
    flye_asm_coverage = null

    isolates = true
    resfinder_threshold = "0.8"
    resfinder_coverage = "0.6"

    monochrome_logs = false
    validate_params = true
    show_hidden_params = false
    analyse_unclassified = false
    schema_ignore_params = 'show_hidden_params,validate_params,monochrome_logs,aws_queue,aws_image_prefix,wf'
```

```
                    "description": "A single sample name for non-multiplexed data. Permissible if passing a single .fastq(.gz) file or directory
of .fastq(.gz) files."
                }
            }
        },
        "isolate_options": {
            "title": "Isolate options",
            "type": "object",
            "fa_icon": "fas fa-arrow-left",
            "description": "Parameters relating to antimicrobial resistance analysis with ResFinder.",
            "properties": {
                "isolates": {
                    "type": "boolean",
                    "title": "Isolates mode",
                    "default": true,
                    "description": "Run the Isolates pipeline on the assembly results if set to True.",
                    "help_text": "Isolates mode adds further analysis options to the workflow such as multi-locus sequence typing and antimicrobial
resistance calling, as well as producing single reports for each sample in the run."
                },
                "resfinder_threshold": {
                    "type": "string",
                    "title": "Resfinder gene identity threshold",
                    "default": "0.8",
                    "description": "Threshold of required identity to report a match between a gene in the ResFinder database and the assembly. Valid
interval: 0.00-1.00",
                    "help_text": "Identity refers to the ratio of base pairs that match between the sequence in your assembly and that of the sequence in
the ResFinder database. Increasing the threshold will results in fewer, but more accurate hits against the database."
                },
                "resfinder_coverage": {
                    "type": "string",
                    "title": "Resfinder gene coverage threshold",
                    "default": "0.6",
                    "description": "Minimum coverage (breadth-of) threshold required to report a match between a gene in the ResFinder database and the
assembly. Valid interval: 0.00-1.00",
                    "help_text": "The amount of an AMR gene that has to be present within the assembly as compared to the reference in the ResFinder
database."
                }
            }
```

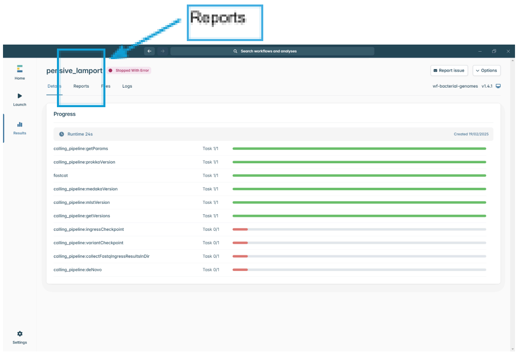
Launch Bacterial Genomes workflow again and check if mlst and resfinder files are generated



Name	Size	Kind
barcode04	- -	Folder
barcode04-isolate-report.html	2.38 MB	Report
barcode04.flye_stats.tsv	239 B	TSV File
barcode04.medaka.fasta	- -	Folder
barcode04.medaka.fasta.gz	1.49 MB	Compressed FASTA File
barcode04.mlst.json	340 B	JSON File
barcode04.prokka.gbk	11.6 MB	Document
barcode04.prokka.gff	6.91 MB	Document
barcode04_resfinder_results	- -	Folder
checkpoints.json	1.66 kB	JSON File
execution	- -	Folder
params.json	1.9 kB	JSON File
results.json	2.47 MB	JSON File
versions.txt	114 B	Plain Text
wf-bacterial-genomes-report.html	6.39 MB	Report

2. *Troubleshooting; Workflow stops with notification that not enough memory is available for workflow, while computer/laptop has the prescribed minimal 32gb RAM*

If workflow "Stopped with error", go to "Reports"

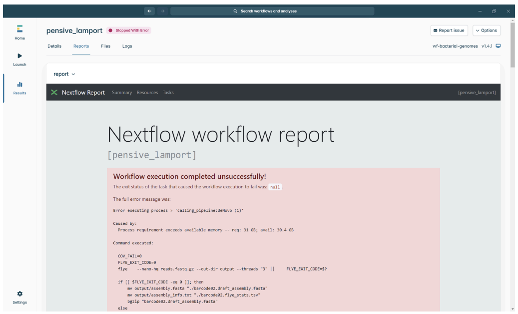


calling_pipeline:getParams	Task 1/1
calling_pipeline:prokkaVersion	Task 1/1
fastcat	Task 1/1
calling_pipeline:medakaVersion	Task 1/1
calling_pipeline:mlstVersion	Task 1/1
calling_pipeline:getVersions	Task 1/1
calling_pipeline:ingressCheckpoint	Task 0/1
calling_pipeline:variantCheckpoint	Task 0/1
calling_pipeline:collectFastqIngressResultsInDir	Task 0/1
calling_pipeline:deNovo	Task 0/1

Check error.
If de novo assembly has stopped and "Caused by: Process requirement exceeds available memory"...

...continue with following steps

If other error is reported, do not continue with following steps



Nextflow workflow report
[pensive_lamport]

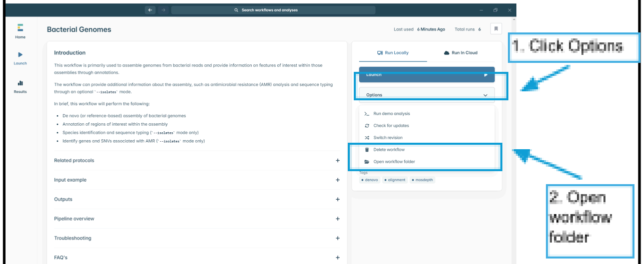
Workflow execution completed unsuccessfully!
The exit status of the task that caused the workflow execution to fail was: `null`.

The full error message was:

```
Error executing process > 'calling_pipeline:deNovo (1)'

Caused by:
  Process requirement exceeds available memory -- req: 31 GB; avail: 30.4 GB

Command executed:

  COV_FAIL=0
  FLYE_EXIT_CODE=0
  flye    --nano-hq reads.fastq.gz --out-dir output --threads "3" ||     FLYE_EXIT_CODE=$?

  if [[ $FLYE_EXIT_CODE -eq 0 ]]; then
      mv output/assembly.fasta "./barcode02.draft_assembly.fasta"
      mv output/assembly_info.txt "./barcode02.flye_stats.tsv"
      bgzip "barcode02.draft_assembly.fasta"
  else
```

Navigate back to Bacterial Genomes Workflow

3. Click "Options"
4. Click "Open workflow folder"



Introduction

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The workflow can provide additional information about the assembly, such as antimicrobial resistance (AMR) analysis and sequence typing through an optional `--isolates` mode.

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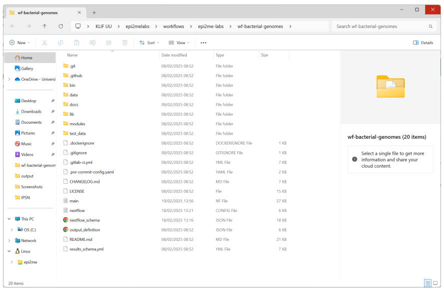
- Annotation of regions of interest within the assembly
- Species identification and sequence typing (`--isolates` mode only)
- Identify genes and SNVs associated with AMR (`--isolates` mode only)

Open file “main” in text editor



Name	Date modified	Type	Size
.git	08/02/2025 08:52	File folder	
.github	08/02/2025 08:52	File folder	
bin	08/02/2025 08:52	File folder	
data	08/02/2025 08:52	File folder	
docs	08/02/2025 08:52	File folder	
lib	08/02/2025 08:52	File folder	
modules	08/02/2025 08:52	File folder	
test_data	08/02/2025 08:52	File folder	
.dockerignore	08/02/2025 08:52	DOCKERIGNORE File	1 KB
.gitignore	08/02/2025 08:52	GITIGNORE File	1 KB
.gitlab-ci.yml	08/02/2025 08:52	YML File	7 KB
.pre-commit-config.yaml	08/02/2025 08:52	YAML File	2 KB
CHANGELOG.md	08/02/2025 08:52	MD File	7 KB
LICENSE	08/02/2025 08:52	File	15 KB
main	19/02/2025 13:56	NF File	27 KB
nextflow	18/02/2025 13:21	CONFIG File	6 KB
nextflow_schema	18/02/2025 13:16	JSON File	18 KB
output_definition	08/02/2025 08:52	JSON File	6 KB
README.md	08/02/2025 08:52	MD File	21 KB
results_schema.yml	08/02/2025 08:52	YML File	7 KB

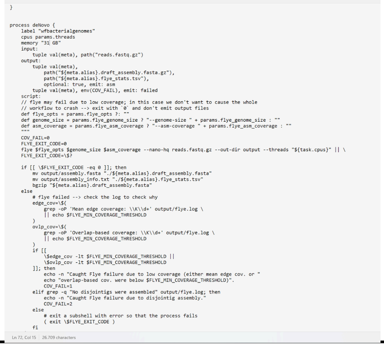
wf-bacterial-genomes (20 items)

Select a single file to get more information and share your cloud content.

20 items

Scroll down to “process deNovo” and change memory “31 GB” to “24 GB”

Save file



```
}

process deNovo {
    label "wfbacterialgenomes"
    cpus params.threads
    memory "31 GB"
    input:
        tuple val(meta), path("reads.fastq.gz")
    output:
        tuple val(meta),
            path("${meta.alias}.draft_assembly.fasta.gz"),
            path("${meta.alias}.flye_stats.tsv"),
            optional: true, emit: asm
        tuple val(meta), env(COV_FAIL), emit: failed
    script:
    // flye may fail due to low coverage; in this case we don't want to cause the whole
    // workflow to crash --> exit with `0` and don't emit output files
    def flye_opts = params.flye_opts ?: ""
    def genome_size = params.flye_genome_size ? "--genome-size " + params.flye_genome_size : ""
    def asm_coverage = params.flye_asm_coverage ? "--asm-coverage " + params.flye_asm_coverage : ""
    """
    COV_FAIL=0
    FLYE_EXIT_CODE=0
    flye $flye_opts $genome_size $asm_coverage --nano-hq reads.fastq.gz --out-dir output --threads "${task.cpus}" || \
    FLYE_EXIT_CODE=\$?

    if [[ \$FLYE_EXIT_CODE -eq 0 ]]; then
        mv output/assembly.fasta "./${meta.alias}.draft_assembly.fasta"
        mv output/assembly_info.txt "./${meta.alias}.flye_stats.tsv"
        bgzip "${meta.alias}.draft_assembly.fasta"
    else
        # flye failed --> check the log to check why
        edge_cov=\$(
            grep -oP 'Mean edge coverage: \\K\\d+' output/flye.log \
            || echo $FLYE_MIN_COVERAGE_THRESHOLD
        )
        ovlp_cov=\$(
            grep -oP 'Overlap-based coverage: \\K\\d+' output/flye.log \
            || echo $FLYE_MIN_COVERAGE_THRESHOLD
        )
        if [[
            \$edge_cov -lt $FLYE_MIN_COVERAGE_THRESHOLD ||
            \$ovlp_cov -lt $FLYE_MIN_COVERAGE_THRESHOLD
        ]]; then
            echo -n "Caught Flye failure due to low coverage (either mean edge cov. or "
            echo "overlap-based cov. were below $FLYE_MIN_COVERAGE_THRESHOLD)".
            COV_FAIL=1
        elif grep -q "No disjointigs were assembled" output/flye.log; then
            echo -n "Caught Flye failure due to disjointig assembly."
            COV_FAIL=2
        else
            # exit a subshell with error so that the process fails
            ( exit \$FLYE_EXIT_CODE )
        fi
```

Ln 72, Col 15 | 26.709 characters

Open file “nextflow” in text editor

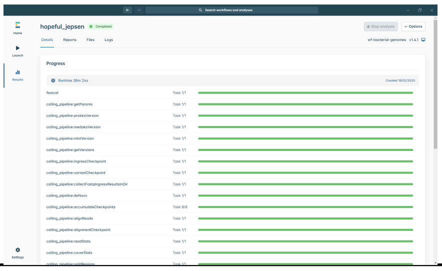
Scroll down to awsbatch process and change
“memory = 31 G” to “memory = 24 G”

Save file

```
    conda {
        conda.enabled = true
    }

    awsbatch {
        process {
            executor = 'awsbatch'
            queue = "${params.aws_queue}"
            memory = '31G'
            withLabel:wf_common {
                container = "${params.aws_image_prefix}-wf-common:${params.wf.common_sha}"
            }
            withLabel:wfbacterialgenomes {
                container = "${params.aws_image_prefix}-wf-bacterial-genomes:${params.wf.container_sha}"
            }
            withLabel:prokka {
                container = "${params.aws_image_prefix}-prokka:${params.wf.container_sha_prokka}-root"
            }
            withLabel:medaka {
                container = "${params.aws_image_prefix}-medaka:${params.wf.container_sha_medaka}"
            }
            withLabel:amr {
                container = "${params.aws_image_prefix}-resfinder:${params.wf.container_sha_resfinder}"
            }
            withLabel:mlst {
                container = "${params.aws_image_prefix}-mlst:${params.wf.container_sha_mlst}"
            }
            withLabel:seqsero2 {
                container = "${params.aws_image_prefix}-seqsero2:${params.wf.container_sha_seqsero}"
            }
            shell = ['/bin/bash', '-euo', 'pipefail']
        }
    }
}
```

Launch Bacterial Genomes workflow again and check if it runs without errors till “Completed”



hopeful_jepsen Completed

wf-bacterial-genomes v1.4.1

Progress

Runtime 38m 24s

Created 18/02/2025

fastcat	Task 1/1
calling_pipeline:getParams	Task 1/1
calling_pipeline:prokkaVersion	Task 1/1
calling_pipeline:medakaVersion	Task 1/1
calling_pipeline:mlstVersion	Task 1/1
calling_pipeline:getVersions	Task 1/1
calling_pipeline:ingressCheckpoint	Task 1/1
calling_pipeline:variantCheckpoint	Task 1/1
calling_pipeline:collectFastqIngressResultsInDir	Task 1/1
calling_pipeline:deNovo	Task 1/1
calling_pipeline:accumulateCheckpoints	Task 8/8
calling_pipeline:alignReads	Task 1/1
calling_pipeline:alignmentCheckpoint	Task 1/1
calling_pipeline:readStats	Task 1/1
calling_pipeline:coverStats	Task 1/1