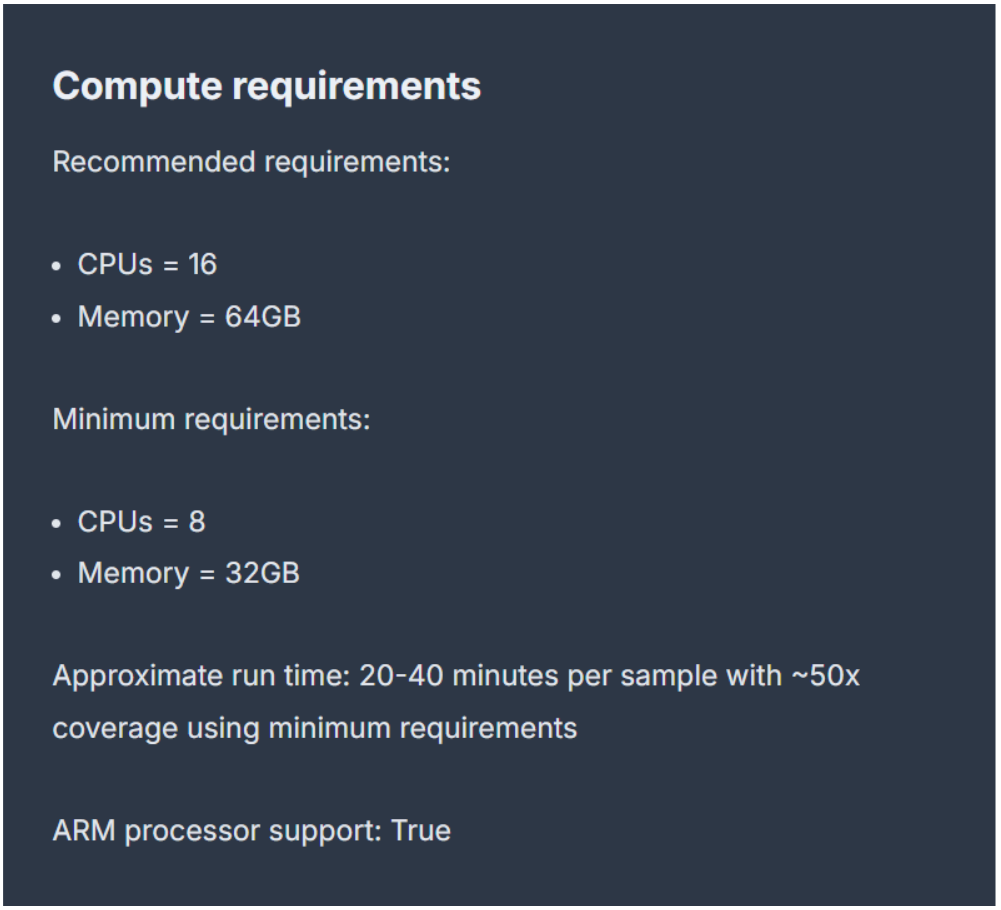
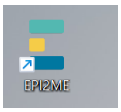
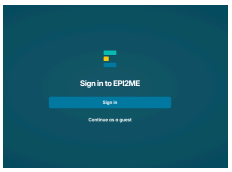
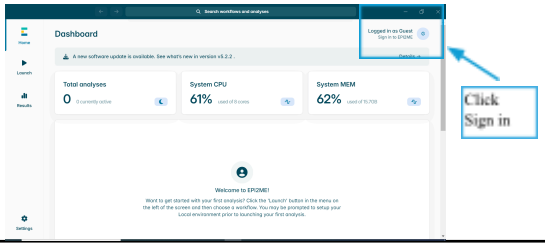
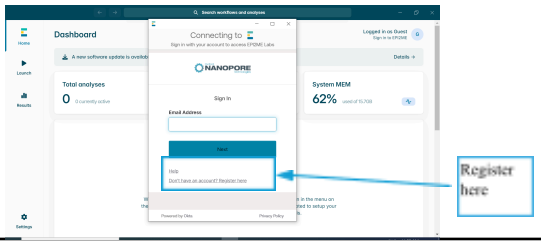
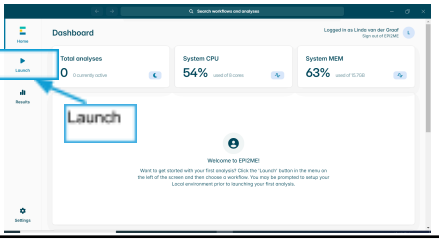
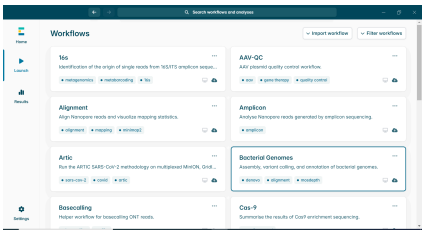
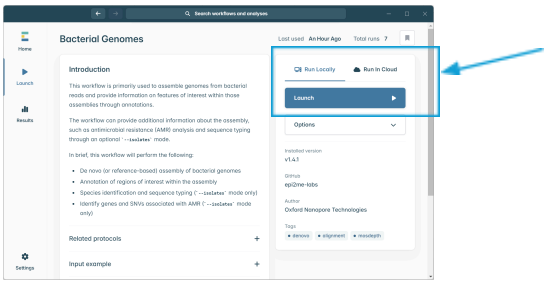
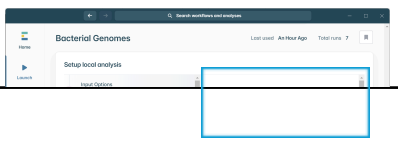
Analysis of Nanopore MinION sequencing data with EPI2ME Bacterial assembly and annotation workflow on Windows computer

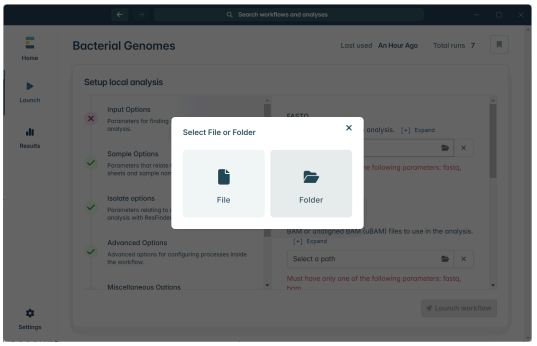
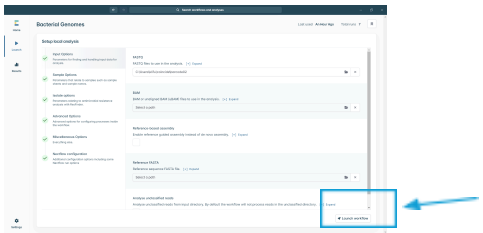
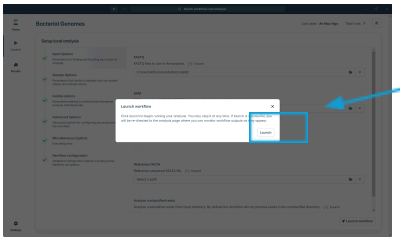
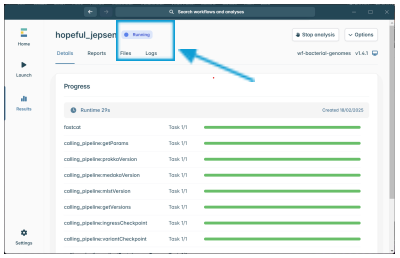
Version: March 2025

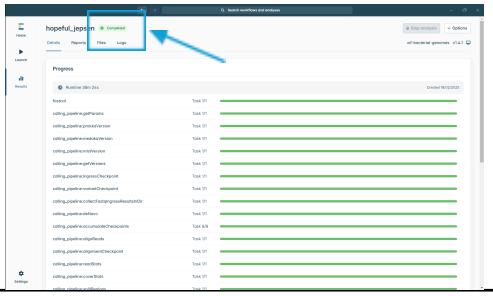
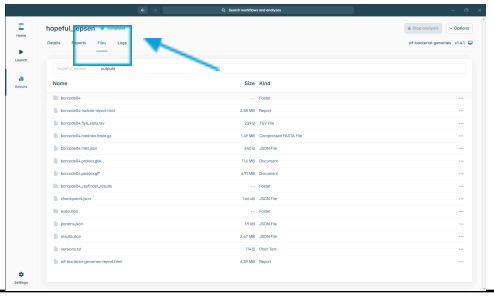
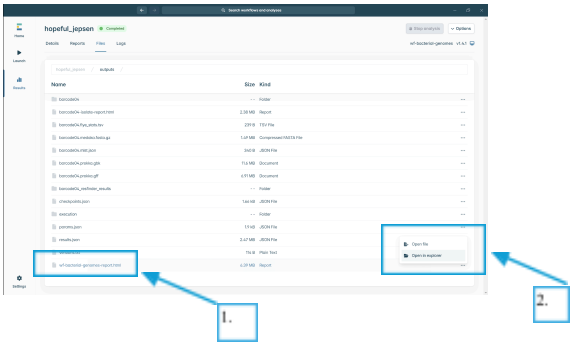
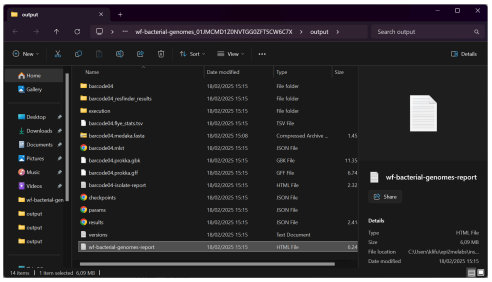
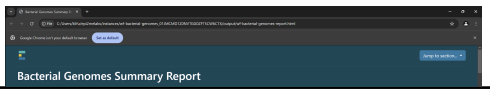
Minimal Requirements for Computer/Laptop to run Epi2ME analysis workflow:

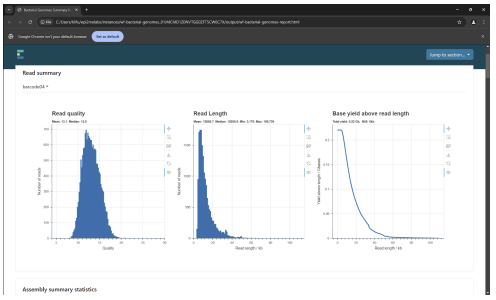
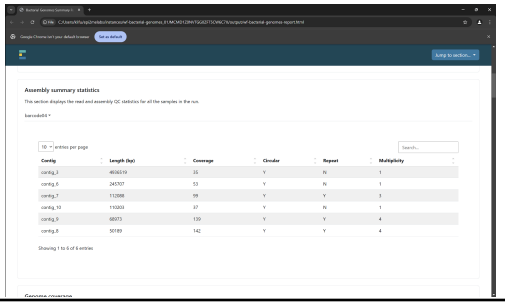
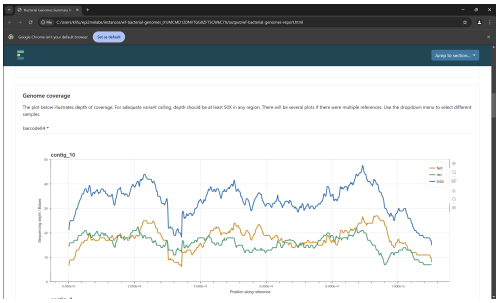
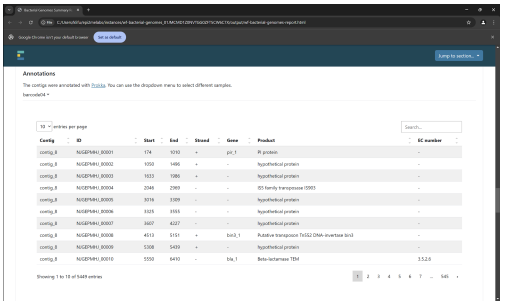
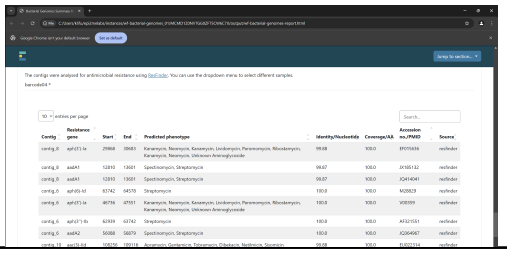


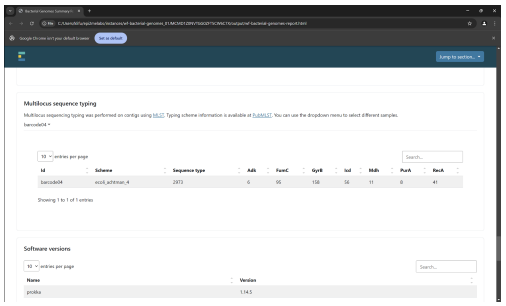
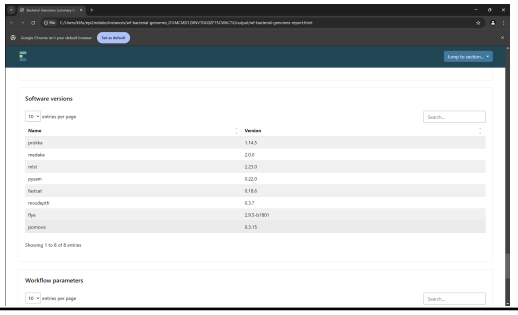
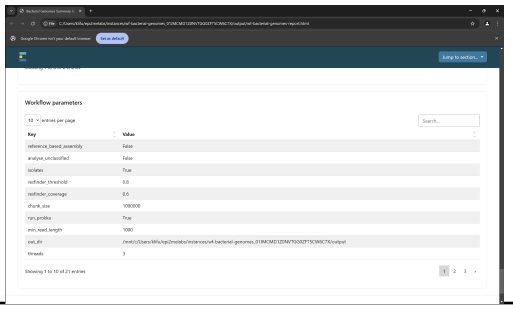
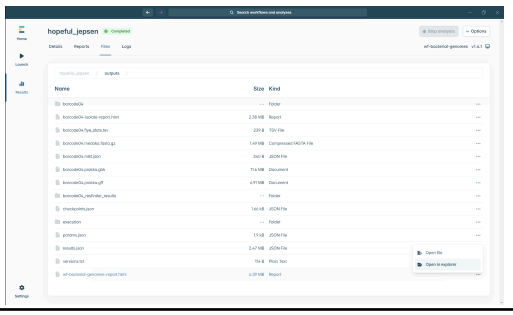
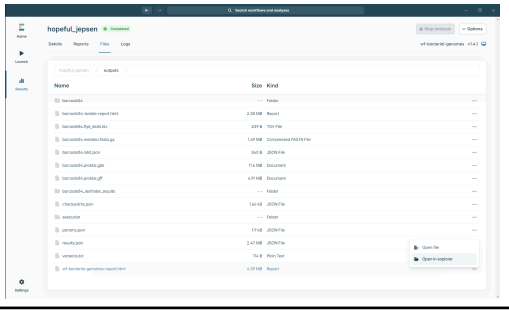
Manual for analysis of Nanopore MinION sequencing data with Epi2ME software on Windows computer

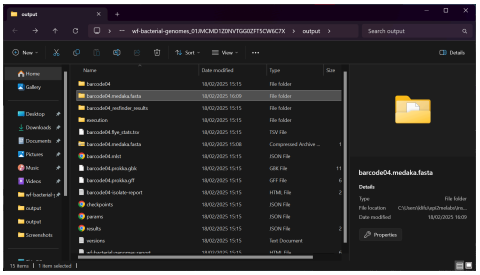
Open EPI2ME Software and Sign in If you do not have an account, click “Continue as a guest”	 
As guest: Click “Sign in to EPI2ME”	
As guest: Click “Don’t have an account? Register here” Register and login	
After login, click “Launch”	
Click on “Bacterial Genomes”	
If the software is correct installed, the “Launch” button will be dark blue. If the software is not correct installed, the red button “Setup required” will be visual. In this case, please install EPI2ME software as described in Manual “Install EPI2ME Software”	
Click on “Launch” button	

At FASTQ section, click on "Select a path"	
Click on Folder and navigate to folder with Nanopore Sequence Data Select Folder "fastq_passed" Note 1: the complete folder with all barcodes can be processed in 1 run. This will take several hours. Note 2: We had an error once, and the whole run was stopped due to a error (number 1) of Flye of an empty barcode. The solution was to delete the empty barcode folders in de "fastq_passed" folders.	
Do not select BAM, reference-based assembly and Reference fasta files Click "Launch Workflow"	
Click "Launch" and workflow will start	
Progress screen with running analysis	

Example of completed analysis	
Navigate to “Files”	
1. Go to file “wf_bacterial_genomes_report.html” 2. Open file in Explorer	
In Explorer: 1. right mouse click on wf-bacterial-genomes-report.html file 2. select “open with” 3. select browser, for example google chrome	
	

Bacterial Genomes Analysis Summary report is shown in browser	
Asses the read summary. Read length should be at least 2kb	
Assembly summary statistics Multiplicity is how many times a particular edge appears in the genome (it is calculated based on coverage)	
Coverage per contig can be assessed	
Annotations Gff and gbk files generated by Prokka also available in files in “outputs” folder	
Resfinder output to assess antimicrobial resistance genes Resfinder results are also available as text file in “outputs” folder (*_resfinder_results)	

MLST Sequence Type result	
Used software versions for analysis with Epi2me	
Workflow parameters	
In “outputs” folder: gff and gbk files are files with annotations	
In “outputs” folder: *.medaka.fasta.gz is assembled fasta file	

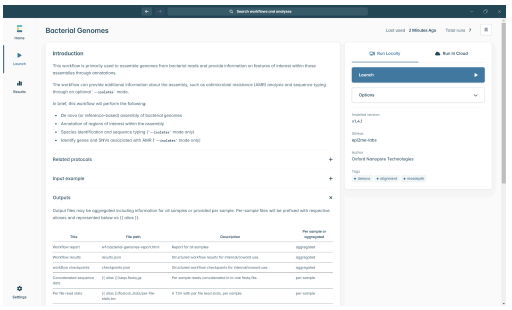
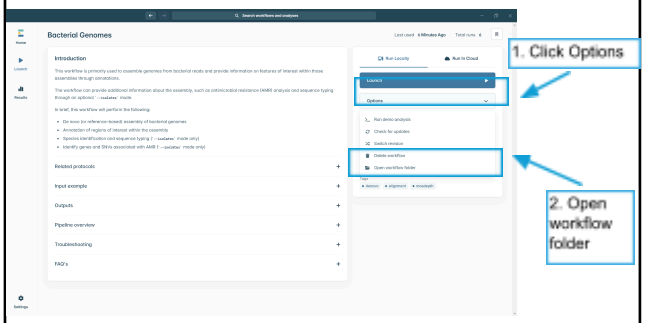
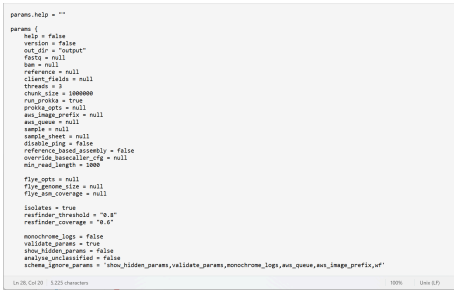
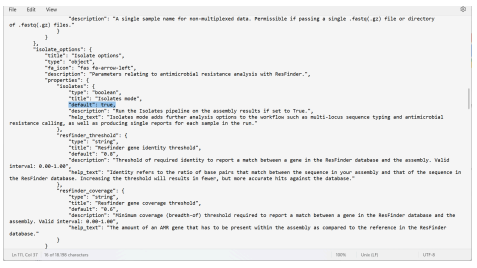
In explorer: - right mouse click on barcode*.medaka.fasta.gz - select Extract All	
Extract generates new folder with assembled *.fasta file	

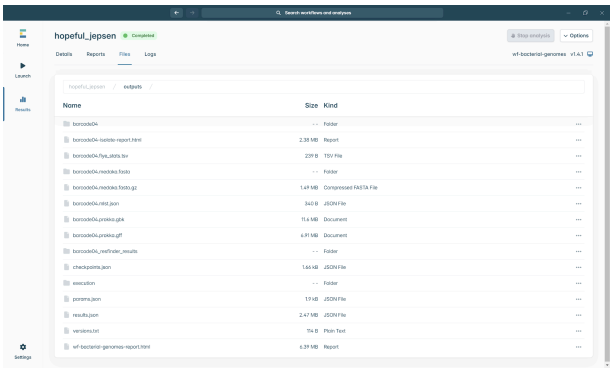
ADVANCED EPI2ME installation options – change Workflow files

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

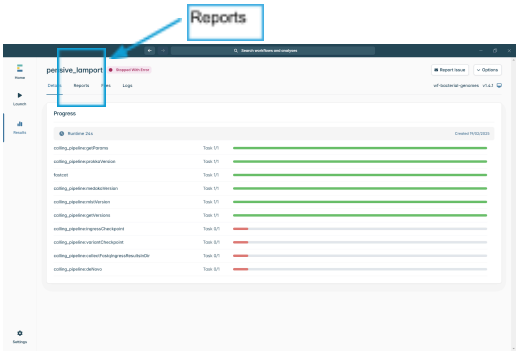
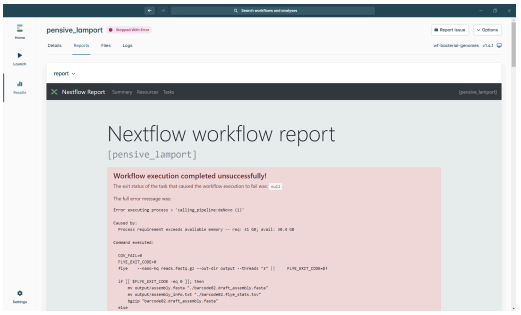
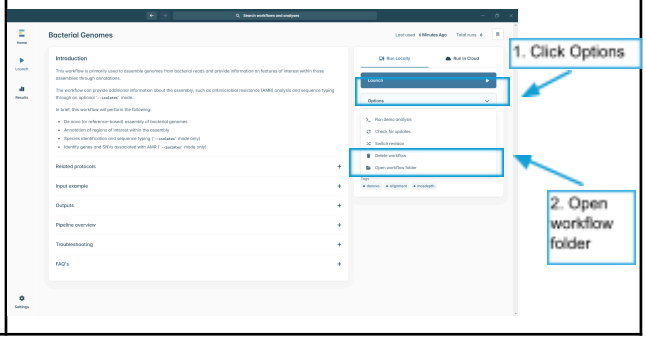
1. Advanced installation; change workflow to “isolates mode”. Default setting is that isolates mode of bacterial genomes workflow it turned off. This isolates mode will add analysis like species identification, MLST STs and Resfinder result.

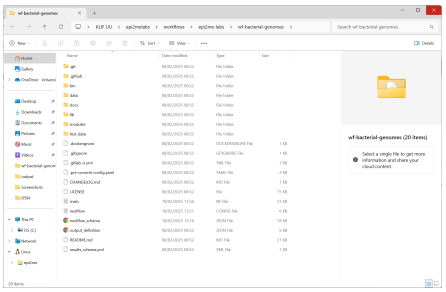
Follow the following steps to turn isolates mode on.

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” in text editor Change: “isolates = false” in “isolates = true” Save file	
Open file “nextflow_scheme” in text editor Change for isolates mode: “"default": false,” in "default": true,” Save file	

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

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”	
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	
Navigate back to Bacterial Genomes Workflow 3. Click “Options” 4. Click “Open workflow folder”	

Open file “main” in text editor	
Scroll down to “process deNovo” and change memory “31 GB” to “24 GB” Save file	
Open file “nextflow” in text editor Scroll down to awsbatch process and change “memory = 31 G” to “memory = 24 G” Save file	
Launch Bacterial Genomes workflow again and check if it runs without errors till “Completed”