Oxford Nanopore Technologies

EPI2ME

Rapid whole-genome sequencing, *de novo* assembly, and characterisation of bacterial isolates

The nanopore-only microbial isolate sequencing solution (NO-MISS), combined with the EPI2ME™ wf-bacterial-genomes workflow, enables rapid sequencing and automated analysis of bacterial isolates, including efficient *de novo* assembly, identification, sequence typing, and predicted antimicrobial resistance profiling

More information can be found at: **epi2me.nanoporetech.com/epi2me-docs/workflows/wf-bacterial-genomes**

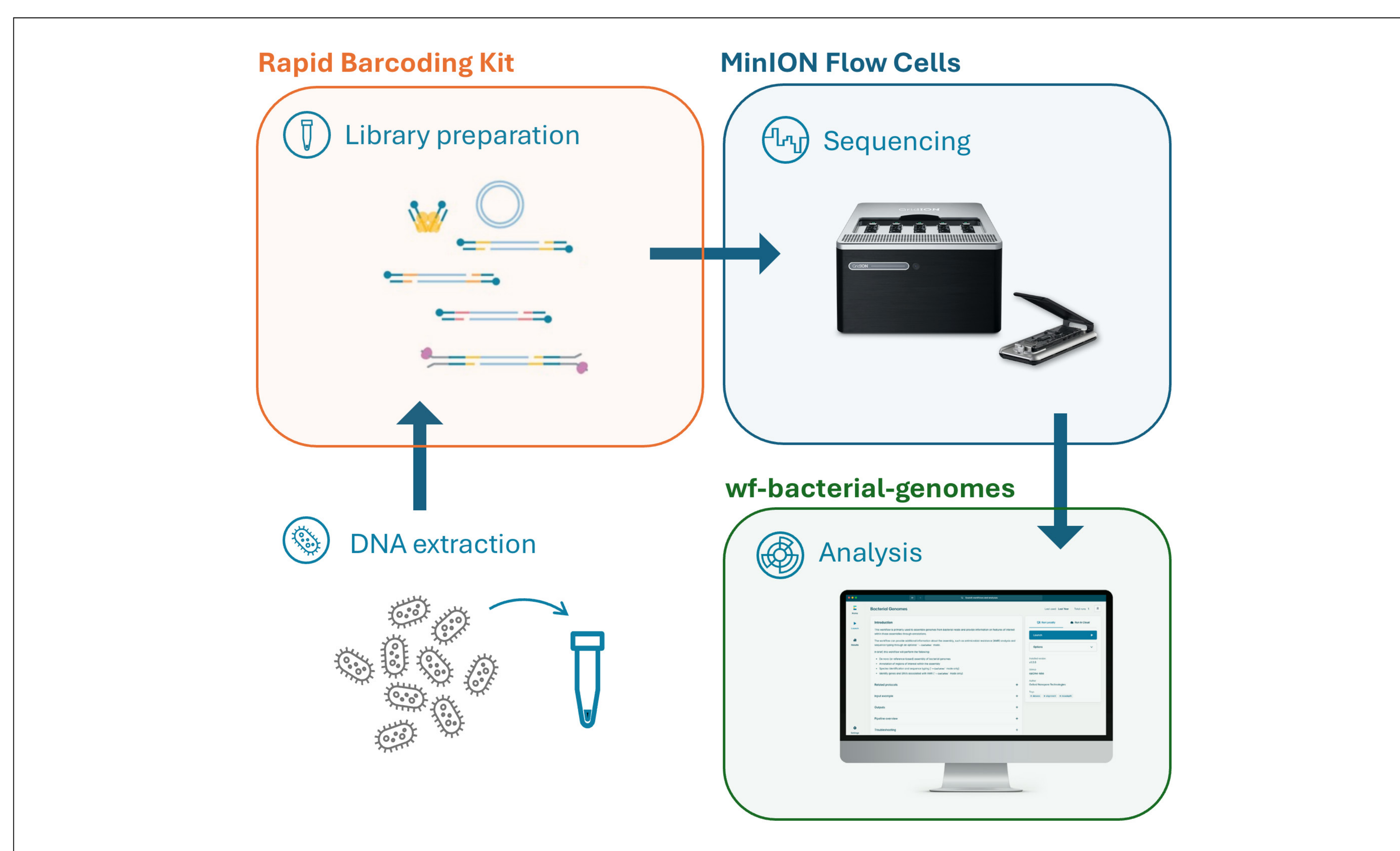


Fig. 1 Overview of the end-to-end process for characterisation of bacterial isolates.

Bacterial isolates characterisation

Whole-genome sequencing and characterisation of microbial isolates is a valuable approach across many settings, including in public health, food safety, biopharma, and research sectors. In particular, predicting the antimicrobial resistance status or other phenotypic properties of strains is important, especially during outbreaks. Oxford Nanopore sequencing overcomes limitations that exist for short-read bacterial sequencing, and enables high-quality, contiguous genome assemblies that resolve complex genomic structures, including repetitive elements and plasmids. Here we demonstrate the nanopore-only microbial isolate sequencing solution (NO-MISS) approach, combined with analysis using the wf-bacterial-genomes workflow, on 32 foodborne pathogens from an FDA proficiency panel.

Fig. 3 a) The EPI2ME platform was used to run the wf-bacterial-genomes workflow and generate a report. The report includes b) quality metrics, and c) *de novo* assembly statistics with further characterisation results.

Analysis with wf-bacterial-genomes via EPI2ME

wf-bacterial-genomes can be run either on the command line or via the EPI2ME Desktop Application, which provides a simple graphical interface for setting parameters and running the workflow. It accepts either FASTQ or BAM files as input. In this example, FASTQ files from MinKNOW were used with default parameters, and with additional isolate characterisation analysis selected. The report generated includes quality control metrics and results for each sample, indicating that the sequencing was completed successfully and with high accuracy and coverage.

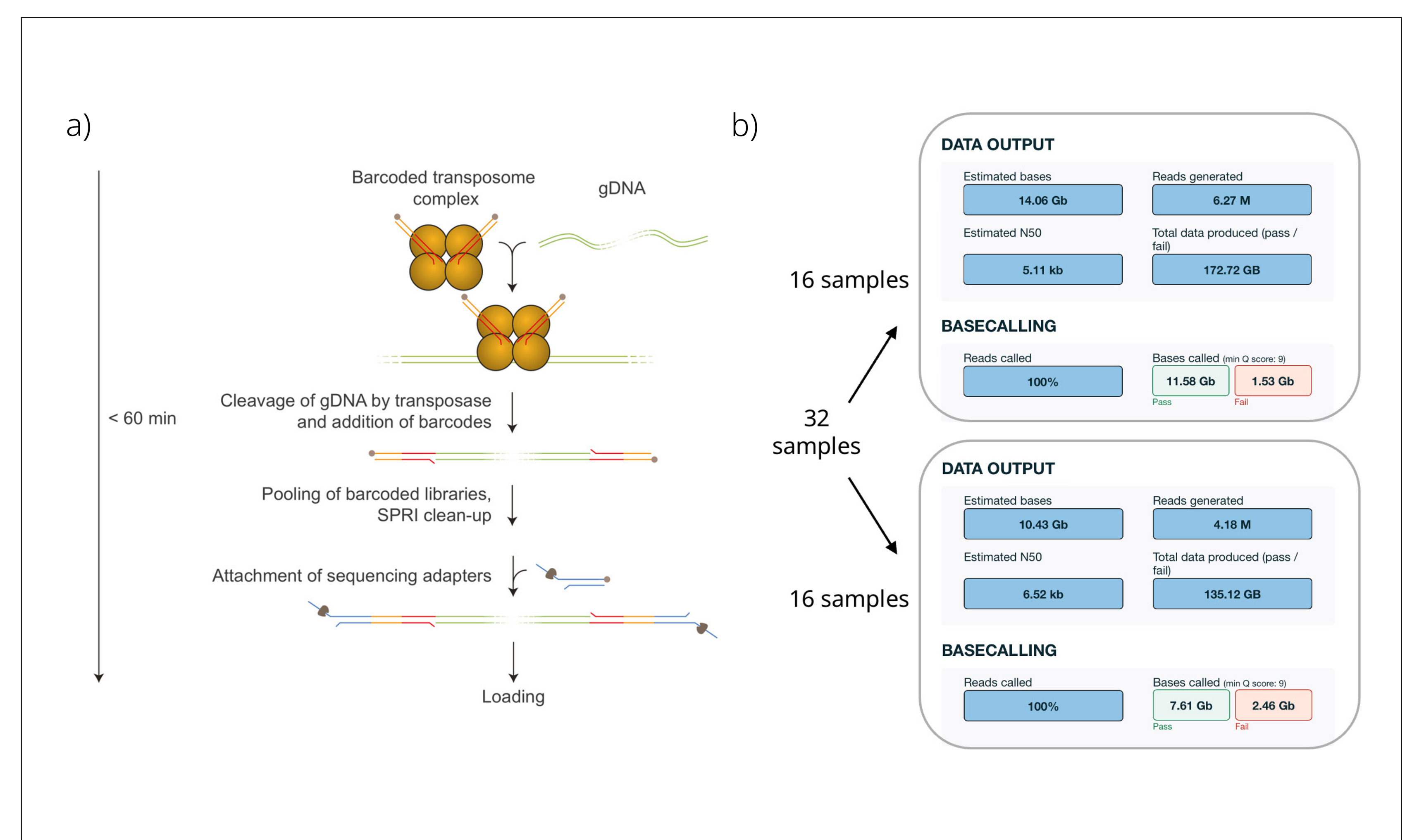
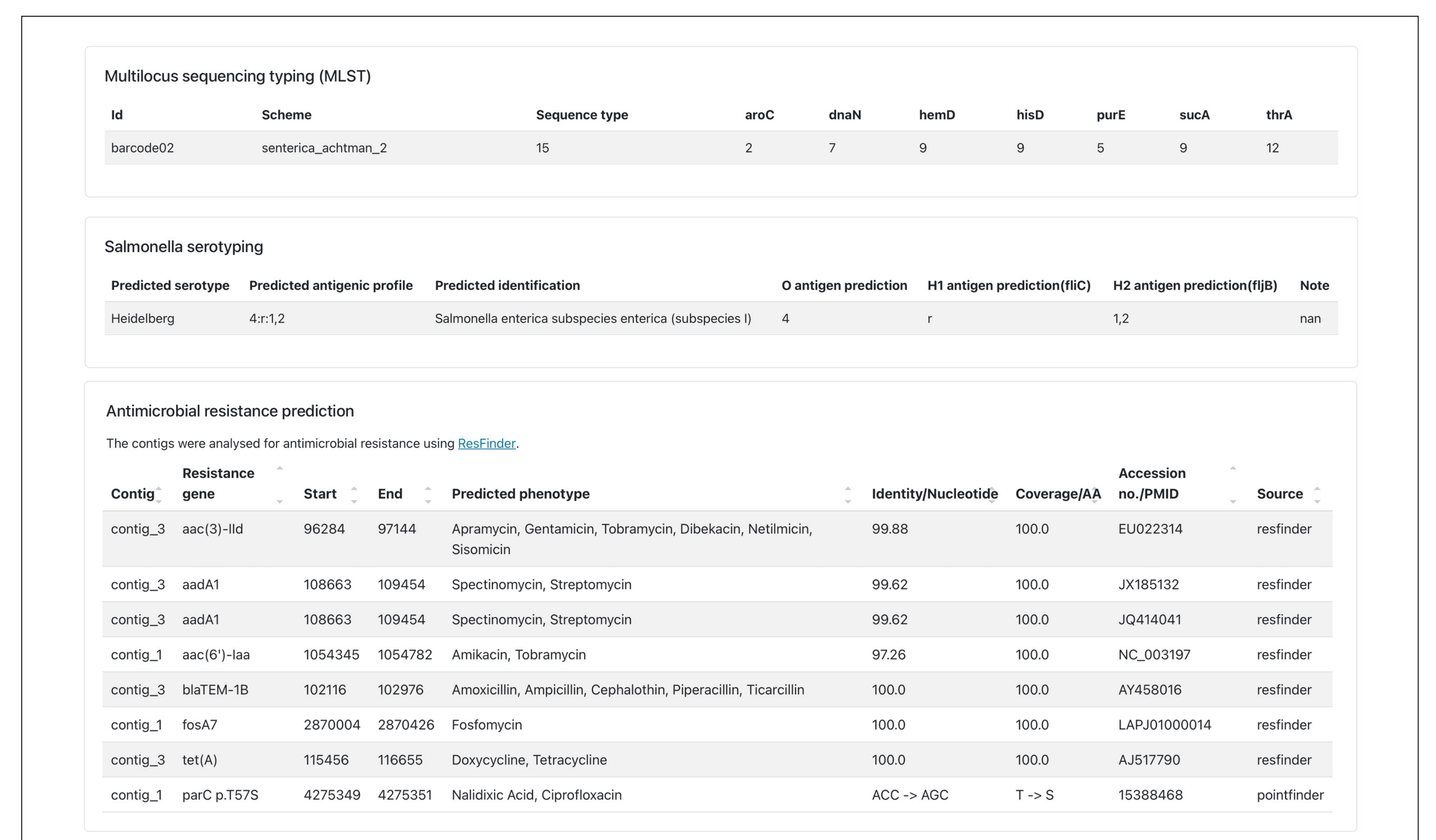


Fig. 2 a) PCR-free library preparation with the Rapid Barcoding Kit. A transposase is used to fragment and attach barcodes to DNA before adding a sequencing adapter. b) Sequencing is carried out on a benchtop GridION™ device using two MinION™ Flow Cells, each run for 72 hours.

Library preparation and sequencing

Following DNA extraction with Maxwell RSC Cultured Cells DNA Kit, libraries were prepared using the Rapid Barcoding Kit (SQK-RBK114.24). NO-MISS allows for up to 24 samples to be multiplexed together, so the 32 samples were split into two sets of 16 samples and loaded onto two MinION Flow Cells. They were sequenced for 72 hours to reach the minimum target coverage of 50x. Basecalling was performed using high accuracy (HAC) mode on MinKNOW™.



Multilocus sequencing typing (MLST)

Id	Scheme	Sequence type	aroC	dnaN	hemD	hisD	purE	sucA	thrA
barcode02	senterica_achtman_2	15	2	7	9	9	5	9	12

Salmonella serotyping

Predicted serotype	Predicted antigenic profile	Predicted identification	O antigen prediction	H1 antigen prediction(fliC)	H2 antigen prediction(fljB)	Note
Heidelberg	4:r:1,2	Salmonella enterica subspecies enterica (subspecies I)	4	r	1,2	nan

Antimicrobial resistance prediction

The contigs were analysed for antimicrobial resistance using ResFinder.

Contig	Resistance gene	Start	End	Predicted phenotype	Identity/Nucleotide	Coverage/AA	Accession no./PMID	Source
contig_3	aac(3)-IId	96284	97144	Apramycin, Gentamicin, Tobramycin, Dibekacin, Netilmicin, Sisomicin	99.88	100.0	EU022314	resfinder
contig_3	aadA1	108663	109454	Spectinomycin, Streptomycin	99.62	100.0	JX185132	resfinder
contig_3	aadA1	108663	109454	Spectinomycin, Streptomycin	99.62	100.0	JQ414041	resfinder
contig_1	aac(6')-Iaa	1054345	1054782	Amikacin, Tobramycin	97.26	100.0	NC_003197	resfinder
contig_3	blaTEM-1B	102116	102976	Amoxicillin, Ampicillin, Cephalothin, Piperacillin, Ticarcillin	100.0	100.0	AY458016	resfinder
contig_1	fosA7	2870004	2870426	Fosfomycin	100.0	100.0	LAPJ01000014	resfinder
contig_3	tet(A)	115456	116655	Doxycycline, Tetracycline	100.0	100.0	AJ517790	resfinder
contig_1	parC p.T57S	4275349	4275351	Nalidixic Acid, Ciprofloxacin	ACC -> AGC	T -> S	15388468	pointfinder

Fig. 4 Multilocus sequence typing (MLST), serotyping, and antimicrobial resistance prediction report.

Further analyses and antimicrobial resistance characterisation

An additional report was generated for each sample showing further analyses. The example in **Fig. 4** was identified by MLST as belonging to the senterica_achtman_2 scheme and therefore further characterised by SeqSero2, which identified the isolate as belonging to the Heidelberg serotype. Additionally, ResFinder was used to identify genes and SNVs associated with antimicrobial resistance, and in this case, identified eight features associated with resistance. These results demonstrate how NO-MISS can be combined with wf-bacterial-genomes to enable efficient, high-resolution characterisation of bacterial isolates.

Information correct at time of publication. May be subject to change.

Oxford Nanopore Technologies, the Wheel icon, AmPORE-TB, EPI2ME, GridION, MinION, MinKNOW, PromethION, P2 Solo, and P2 are registered trademarks or the subject of trademark applications of Oxford Nanopore Technologies plc in various countries. Information contained herein may be protected by copyright, patents or patents pending of Oxford Nanopore Technologies plc. All other brands and names contained are the property of their respective owners. © 2026 Oxford Nanopore Technologies plc. All rights reserved. Oxford Nanopore Technologies products are RUO. Products labelled/branded as Oxford Nanopore Diagnostics may be RUO or may be regulated as in-vitro diagnostic devices in some jurisdictions, please check individual product labelling.

PO_1301(EN)_V2_19May2026