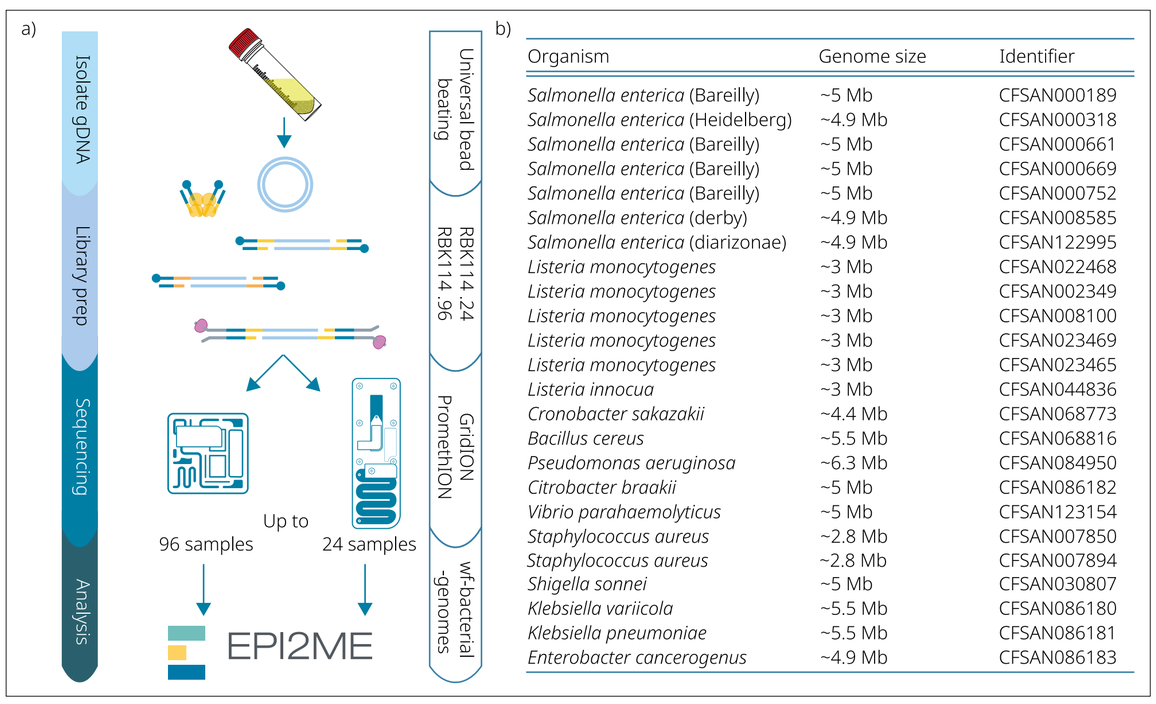
Oxford Nanopore Technologies

Rapid and scalable whole-genome microbial isolate sequencing

The Nanopore-only microbial isolate sequencing solution (NO-MISS), combined with the EPI2ME™ wf-bacterial-genomes workflow, provides a rapid and scalable approach for bacterial whole-genome sequencing and analysis



b)

Organism	Genome size	Identifier
Salmonella enterica (Bareilly)	~5 Mb	CFSAN000189
Salmonella enterica (Heidelberg)	~4.9 Mb	CFSAN000318
Salmonella enterica (Bareilly)	~5 Mb	CFSAN000661
Salmonella enterica (Bareilly)	~5 Mb	CFSAN000669
Salmonella enterica (Bareilly)	~5 Mb	CFSAN000752
Salmonella enterica (derby)	~4.9 Mb	CFSAN008585
Salmonella enterica (diarizonae)	~4.9 Mb	CFSAN122995
Listeria monocytogenes	~3 Mb	CFSAN022468
Listeria monocytogenes	~3 Mb	CFSAN002349
Listeria monocytogenes	~3 Mb	CFSAN008100
Listeria monocytogenes	~3 Mb	CFSAN023469
Listeria monocytogenes	~3 Mb	CFSAN023465
Listeria innocua	~3 Mb	CFSAN044836
Cronobacter sakazakii	~4.4 Mb	CFSAN068773
Bacillus cereus	~5.5 Mb	CFSAN068816
Pseudomonas aeruginosa	~6.3 Mb	CFSAN084950
Citrobacter braakii	~5 Mb	CFSAN086182
Vibrio parahaemolyticus	~5 Mb	CFSAN123154
Staphylococcus aureus	~2.8 Mb	CFSAN007850
Staphylococcus aureus	~2.8 Mb	CFSAN007894
Shigella sonnei	~5 Mb	CFSAN030807
Klebsiella variicola	~5.5 Mb	CFSAN086180
Klebsiella pneumoniae	~5.5 Mb	CFSAN086181
Enterobacter cancerogenus	~4.9 Mb	CFSAN086183

Fig. 1 a) Workflow for 24 or 96 samples and, b) isolates used in this study.

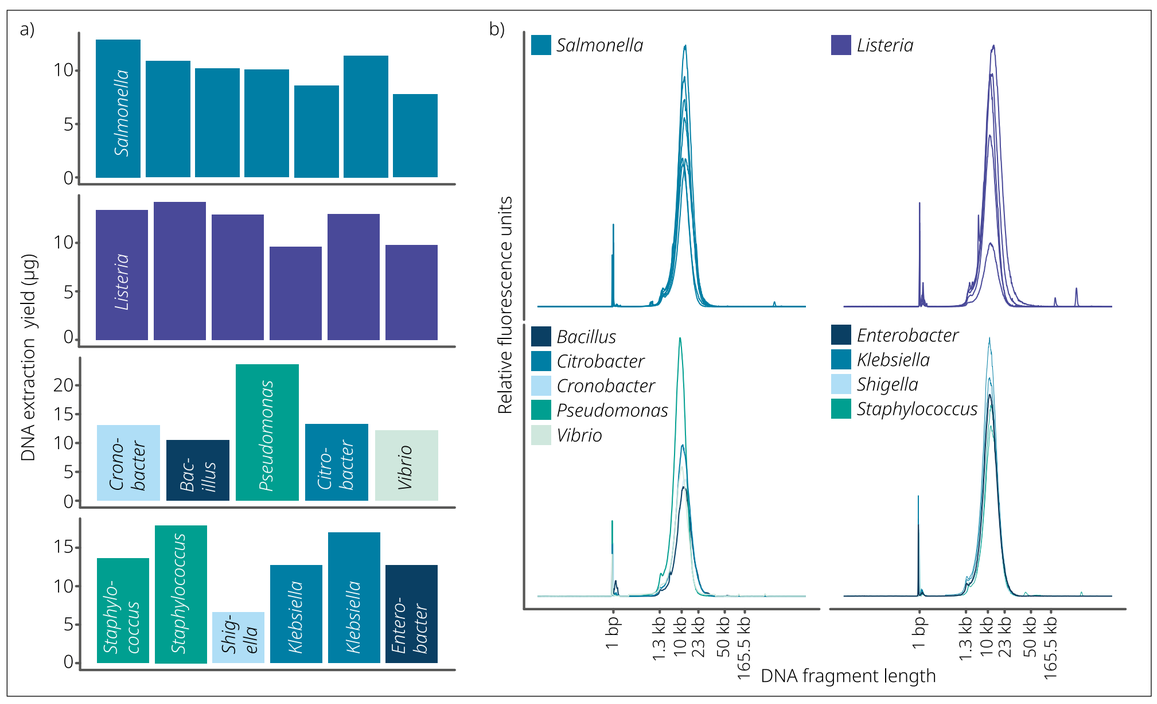


Fig. 2 a) DNA yield from isolates and, b) DNA fragment lengths from isolates.

Extracting DNA from cell cultures for the NO-MISS workflow

NO-MISS is a comprehensive end-to-end protocol designed for flexible bacterial and fungal whole-genome sequencing of up to 96 samples simultaneously on a single flow cell (**Fig. 1a**). DNA extraction methods have been optimised for bacteria and fungi, ensuring optimal yield and performance. In this study, 24 samples spanning a range of bacterial species and genome sizes were selected from the FDA CFSAN panel (**Fig. 1b**). Genomic DNA was extracted using the universal bead-beating method, and libraries were prepared with the Rapid Barcoding Kit 96 V14 (SQK-RBK114.96). Pooled libraries were sequenced on MinION™ or PromethION™ Flow Cells, and the resulting data was analysed using the EPI2ME wf-bacterial-genomes workflow.

Universal bead-beating method yields high-quality genomic DNA suitable for sequencing workflows

Genomic DNA was successfully extracted from 24 bacterial isolates representing 11 species (10 Gram-negative and 1 Gram-positive) using the universal bead-beating method. This approach involves vortex-based bead beating followed by treatment with proteinase K and RNase. The protocol requires 1 ml of overnight liquid culture as input and utilises QIAGEN PowerBead tubes in combination with the MagMAX DNA Multi-Sample Ultra 2.0 Kit. DNA yields ranged from 6.6 to 23.6 µg per sample, well above the 200 ng required for downstream sequencing (**Fig. 2a**). Fragment size analysis using the FemtoPulse system showed a predominant peak above 10 kb, indicating reproducible extraction performance between isolates (**Fig. 2b**).

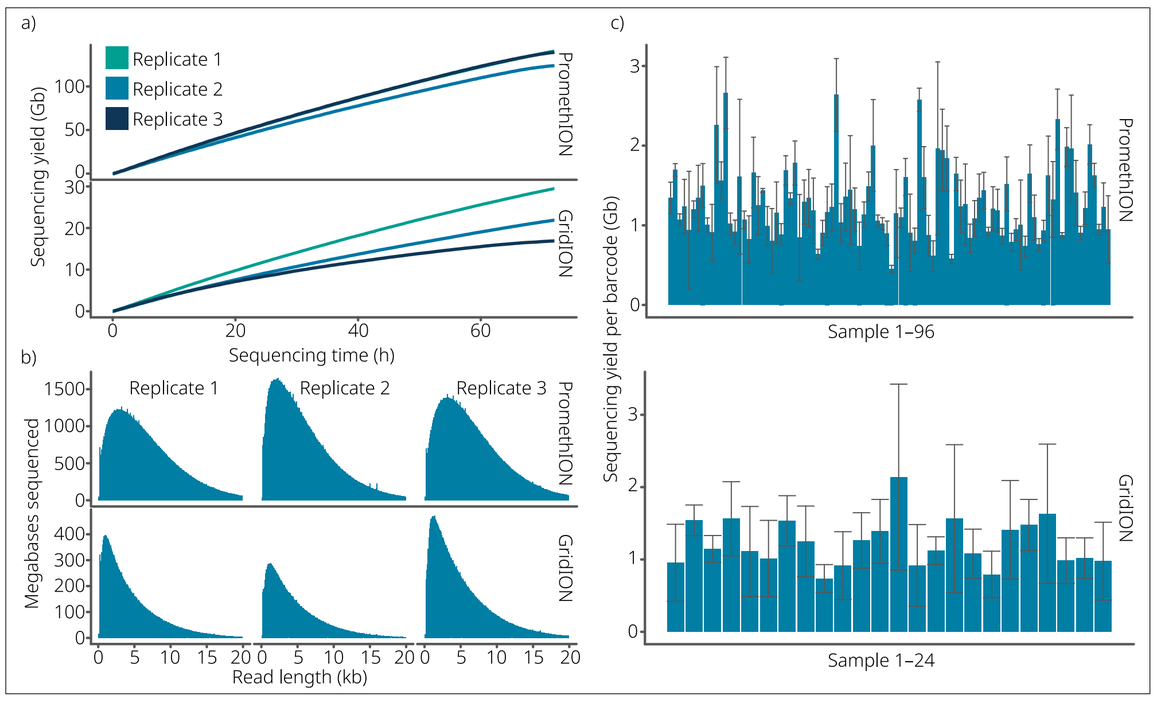


Fig. 3 a) Sequencing yield, b) read length distribution, and c) data per sample.

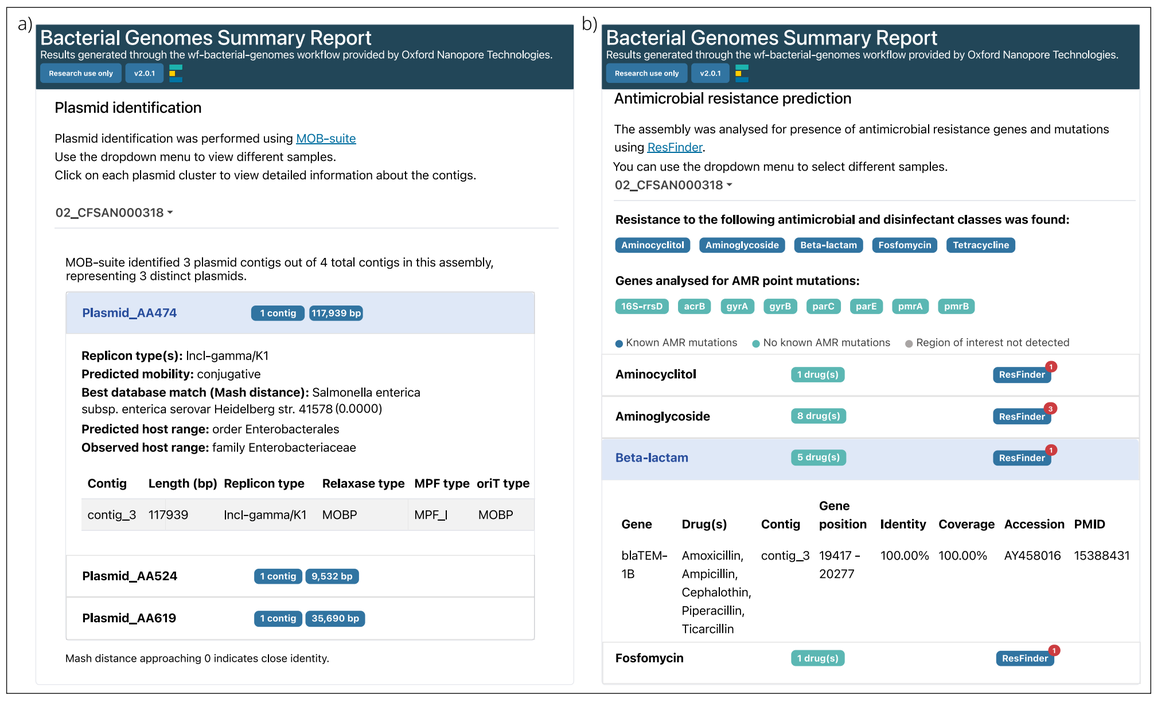


Fig. 4 a) Plasmid identification with MOB-suite and, b) antimicrobial resistance with ResFinder.

Sequencing performance on MinION and PromethION Flow Cells shows high output and reproducibility

To benchmark the NO-MISS protocol, 24 samples (for MinION Flow Cells run on GridION™) and 96 samples (24 samples with four replicates each, for PromethION Flow Cells) were barcoded, pooled, and sequenced for 72 hours in triplicate. For PromethION runs, cumulative output ranged from 123.9 to 140.7 Gb across three flow cells (**Fig. 3a**), with an average read length N50 of 5.37 ±0.48 kb (**Fig. 3b**). Across 96 barcoded samples, the median data output per barcode was 1.2 Gb, with a minimum of 448.1 Mb. For GridION runs, cumulative output ranged from 16.9 to 29.46 Gb, with a mean read length N50 of 3.5 ±0.25 kb. Across the 24 samples, the median data output per barcode was 776.6 Mb, with a minimum output of 215.2 Mb (**Fig. 3c**).

EPI2ME wf-bacterial-genomes identifies plasmids and predicts antimicrobial resistance profiles

Nanopore sequencing data was analysed using the EPI2ME wf-bacterial-genomes workflow (median coverage per sample: GridION = ~144x, PromethION = ~212x). This automated method supports *de novo* or reference-based genome assembly with Flye, followed by variant calling with Medaka and annotation of genomic regions of interest using Bakta. The workflow also enables multilocus sequence typing (MLST), species identification based on genomic similarity using Sourmash and *Salmonella* serotyping with SeqSero2. Recent updates include plasmid identification with MOB-suite (**Fig. 4a**) and antimicrobial resistance gene detection with ResFinder (**Fig. 4b**), with all results incorporated into the final summary report.

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