Rapid reconstruction of multidrug resistant bacterial genomes using CycloneSEQ nanopore sequencing

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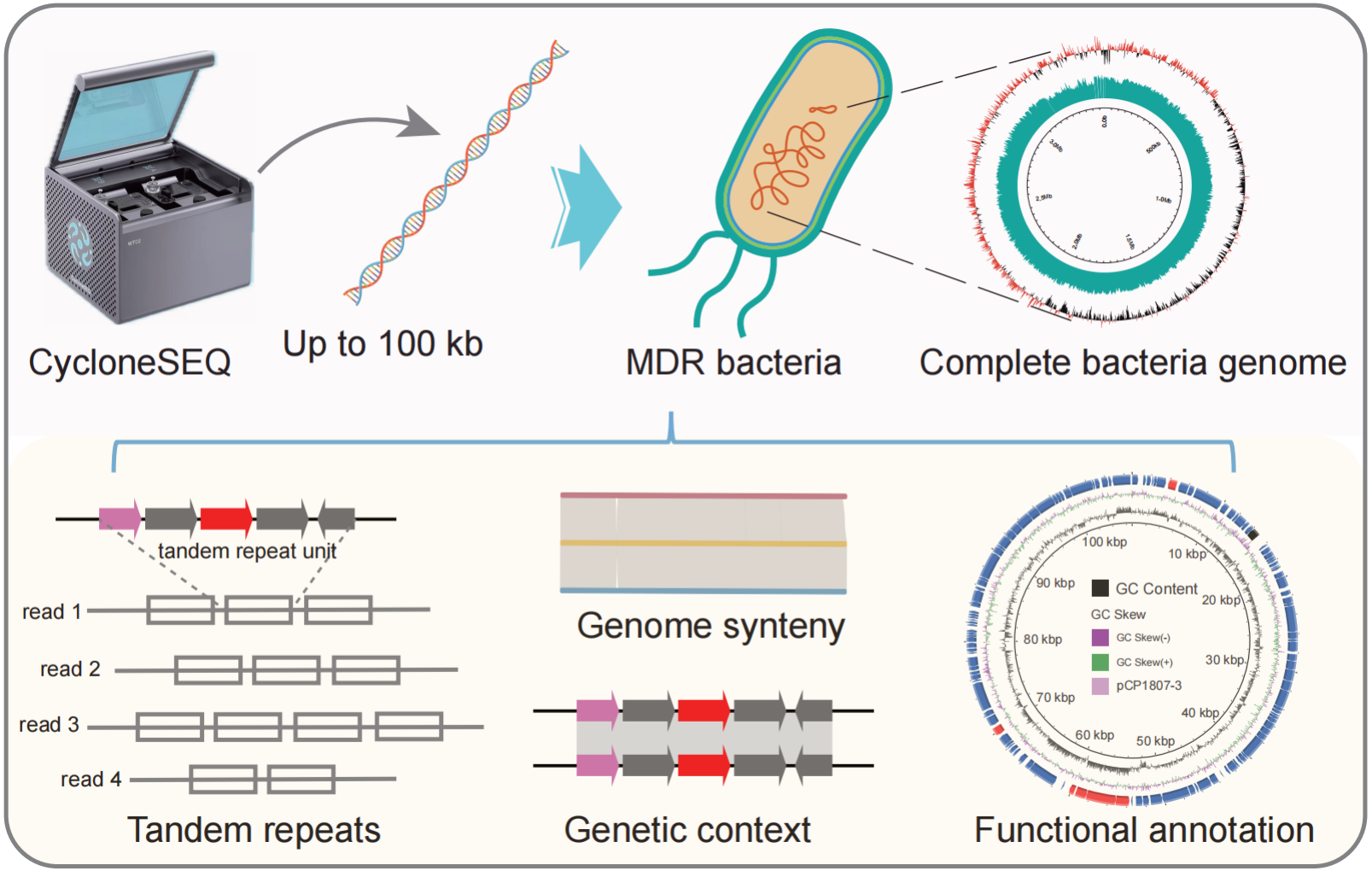
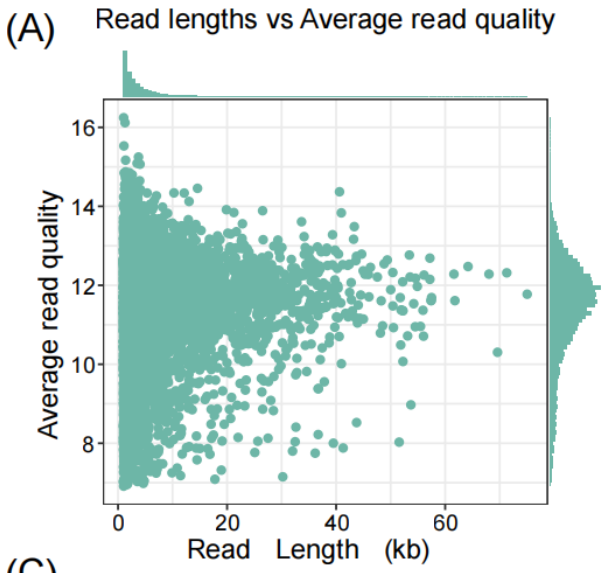
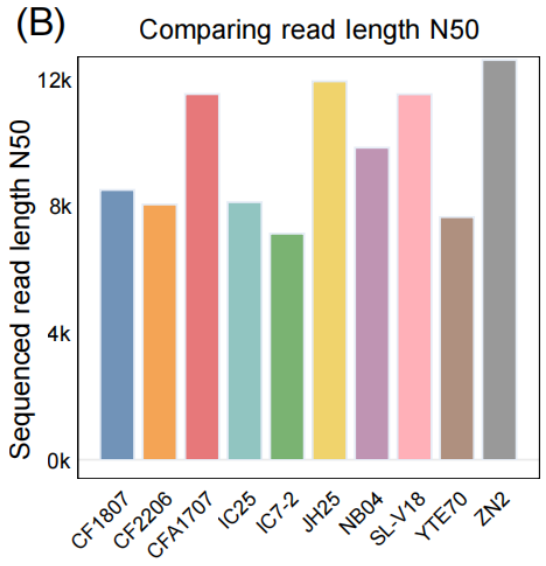
Introduction

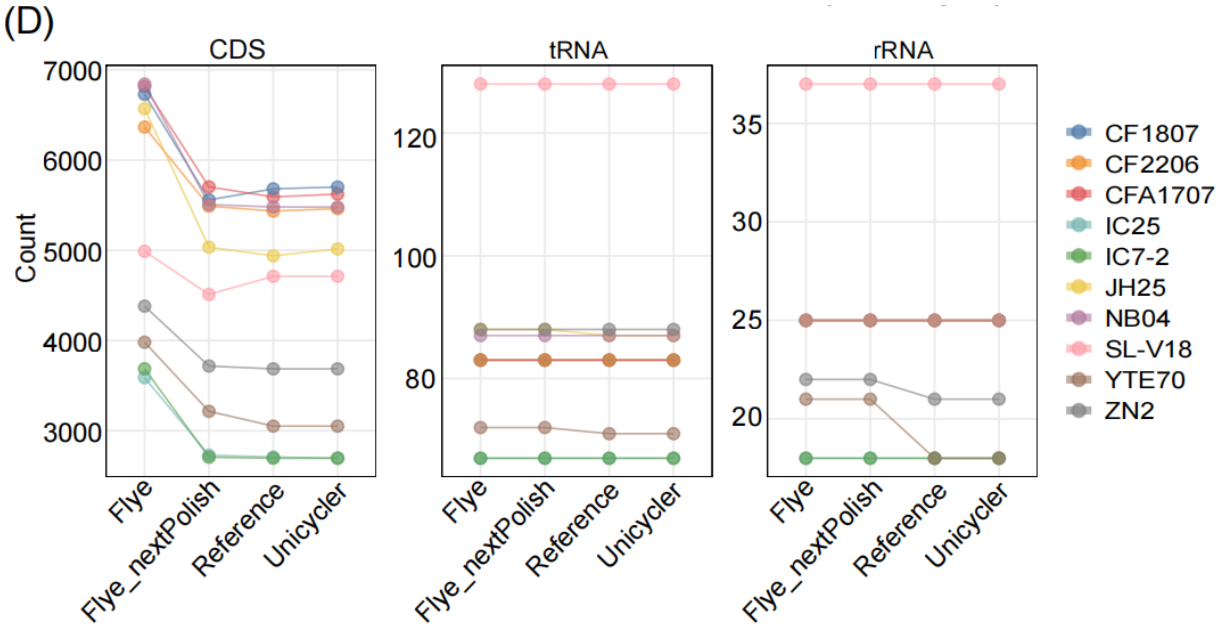


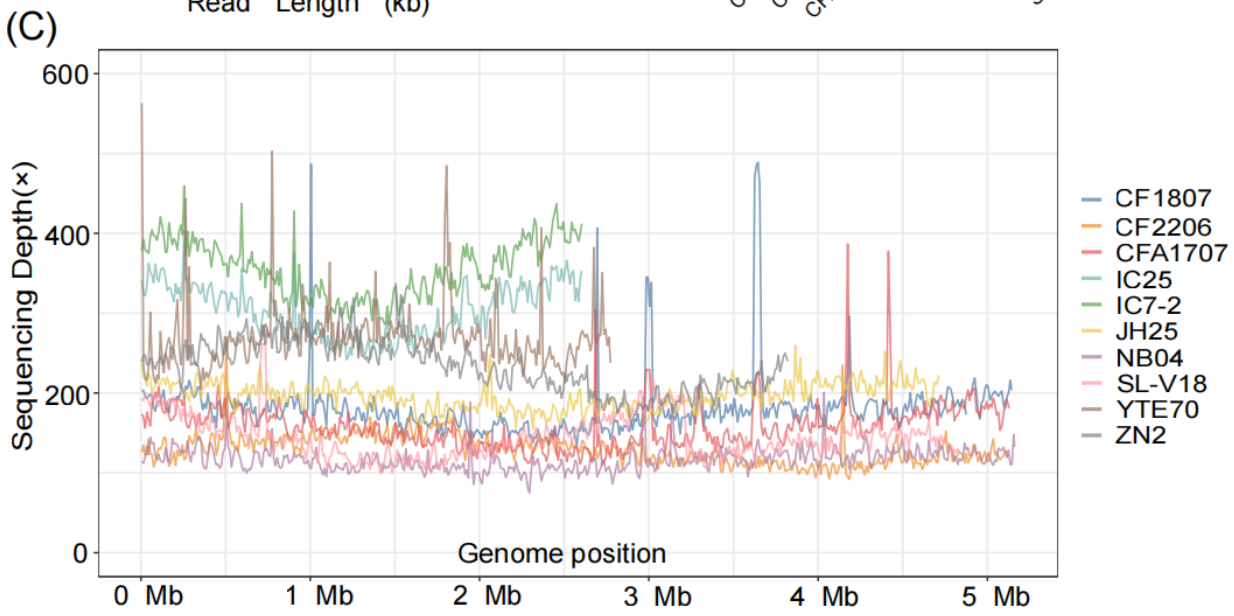
- Multidrug-resistant (MDR) bacteria commonly harbor highly complex genomes enriched with mobile elements and resistance islands, which are challenging to resolve using short-read sequencing;
- The advent of CycloneSEQ enables the generation of complete, highly accurate bacterial genomes, underscoring its value for antimicrobial resistance research and clinical surveillance.

The feature of CycloneSeq data and quality assessment of assembled genomes



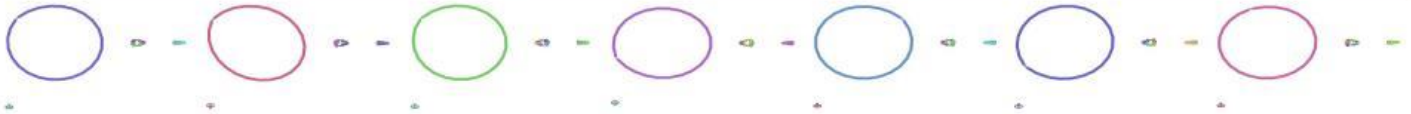






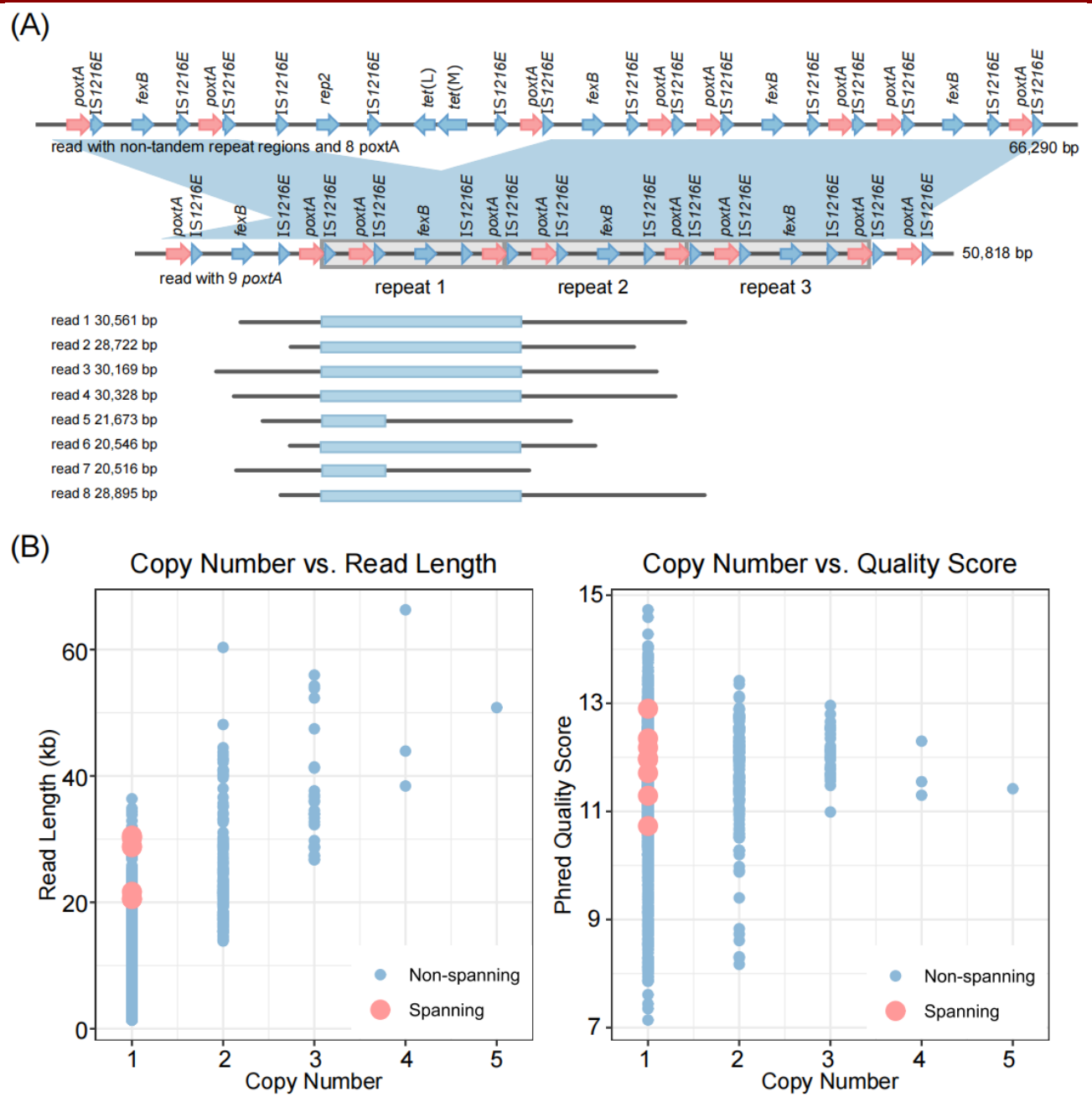
- A large proportion of reads had lengths ranging from 10 to 20 kb, and a subset of exceptionally long reads (> 100 kb) was also detected;
- There were no sequencing gaps and bias in any of the genomes;
- Long-read only assembled genomes resulted in many incorrect predictions for CDS, tRNA, and rRNA.

The influence of sequence depth on the genome assembly and the accuracy of the assembled genome

Depth	100x	50x	30x	20x	15x	10x	5x
flye							
Contigs	7	5	7	5	5	18	59
Misassemblies	3	4	1	4	2	3	4
Misassembled contigs length	30484	53636	21582	53628	35719	1858420	364086
Mismatches per 100 kbp	1.48	2.13	2.98	4.61	9.28	68.81	316.23
Indels per 100 kbp	59.9	43.59	53.72	75.95	109.98	260.88	796.87
Accuracy	99.939%	99.954%	99.943%	99.919%	99.881%	99.670%	98.887%
Misassembled contigs length (nextpolish)	30505	53652	21590	53641	35735	1859761	363771
mismatches per 100 kbp (nextpolish)	1.48	2.06	2.03	2.83	4.25	30.93	45.89
indels per 100 kbp (nextpolish)	0.56	0.98	2.14	4.22	4.67	42.47	69.03
Total length (nextpolish)	2843173	2864705	2855402	2864695	2846617	2869973	2508344
Accuracy (nextpolish)	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Depth	**100x**	**50x**	**30x**	**20x**	**15x**	**10x**	**5x**
unicycler							
Contigs	6	6	6	6	6	6	6
Misassemblies	0	0	0	0	0	0	0
Misassembled contigs length	0	0	0	0	0	0	0
Mismatches per 100 kbp	0	0	0	0	0	0.04	0
Indels per 100 kbp	0	0	0	0	0	0.04	0
Accuracy	100%	100%	100%	100%	100%	99.992%	100%

- Using only nanopore long-read sequencing data at a depth of 15 × could obtain a bacterial genome with an accuracy of 98.88%;
- Beyond a depth of 50 ×, the accuracy of the assembled genomes did not show further improvement;
- A complete bacterial genome could be obtained with a long-read sequencing depth of 5 × using hybrid assembly.

The ability of CycloneSEQ to resolve complex genetic structures of MDR bacteria

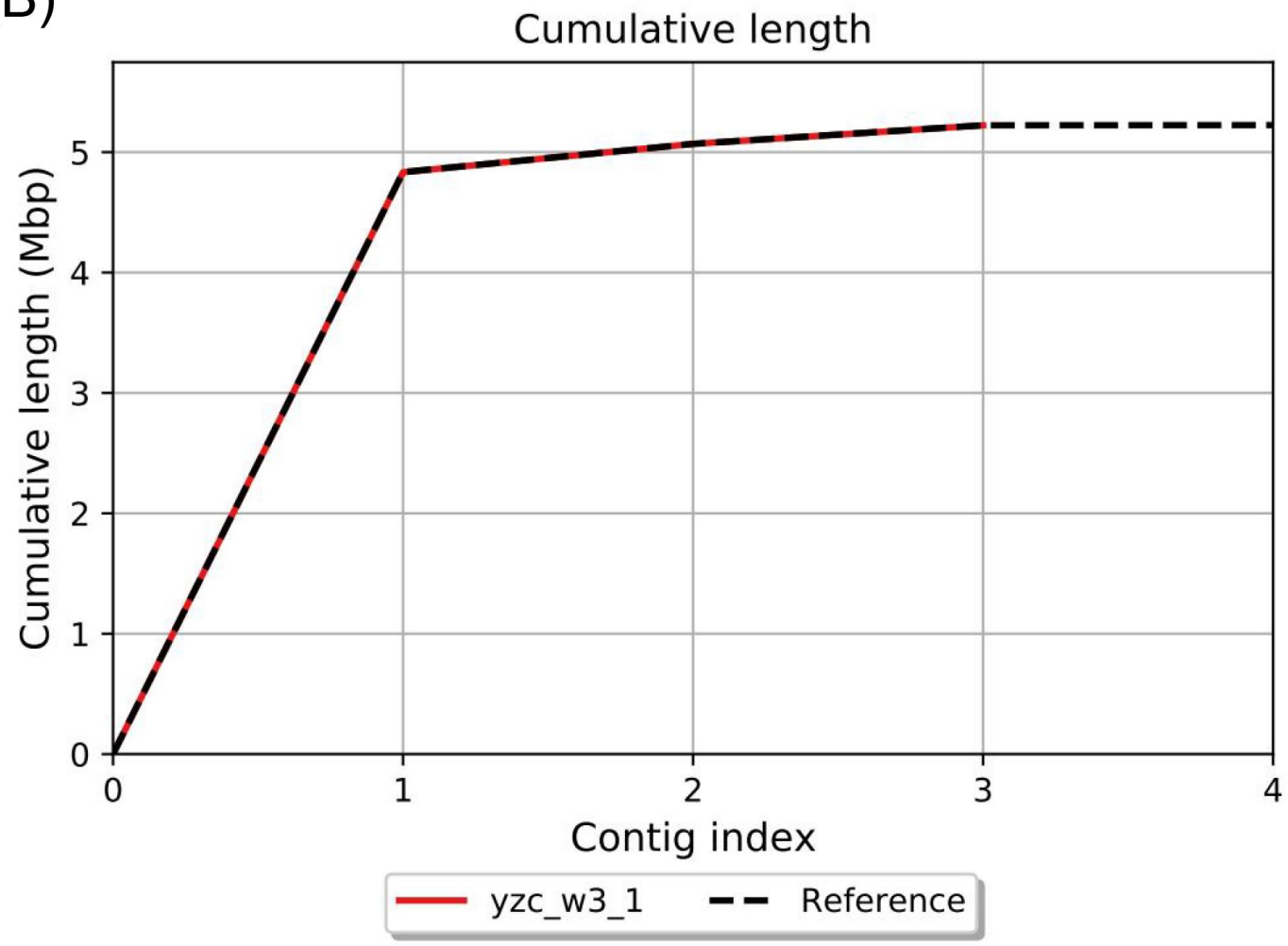


Improvement in the accuracy of cycloneSEQ sequencing

(A)

Genome statistics	yzc_w3_1
Genome fraction (%)	99.969
Duplication ratio	1
Largest alignment	4 833 716
Total aligned length	5 222 882
NGA50	4 833 716
LGA50	1
Misassemblies	
# misassemblies	0
Misassembled contigs length	0
Mismatches	
# mismatches per 100 kbp	8.9
# indels per 100 kbp	14.7
# N's per 100 kbp	0
Statistics without reference	
# contigs	3
Largest contig	4 833 716
Total length	5 222 882
Total length (>= 1000 bp)	5 222 882
Total length (>= 10000 bp)	5 222 882
Total length (>= 50000 bp)	5 222 882

(B)



The genome assembled exclusively with CycloneSEQ reads contained no misassemblies, only a few mismatches, and achieved an overall accuracy of 99.98%.

Summary

- Our study provides a comprehensive evaluation of the CycloneSEQ nanopore sequencing platform for MDR bacterial genome assembly.
- We demonstrated that CycloneSEQ long-read data enable the reconstruction of near-complete bacterial genomes, resolve complex genetic structures, and improve the quality of hybrid assembly.
- Using the updated CycloneSEQ sequencing platform, complete and highly accurate genomes of multidrug-resistant bacteria can be assembled without the need for short-read–based polishing.

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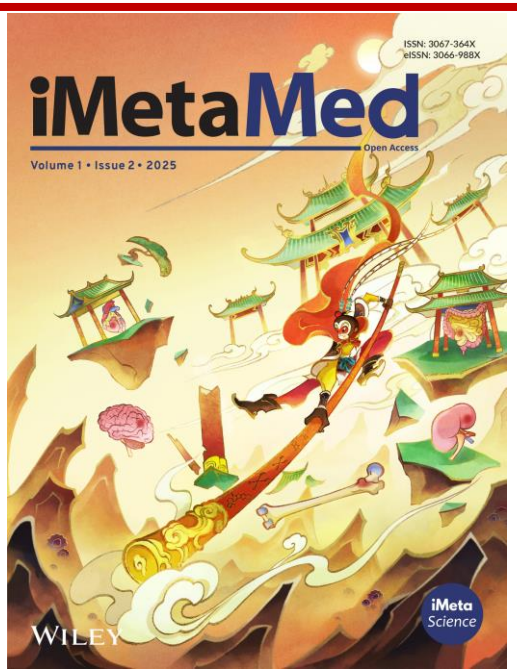

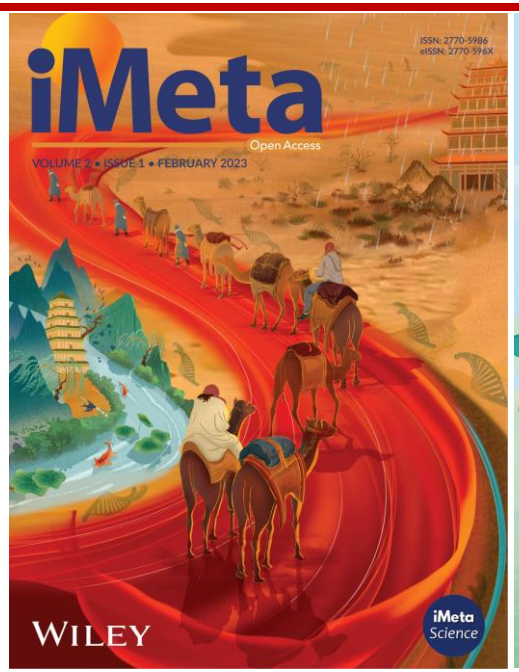

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