Unlocking the potential of rapid, accessible, comprehensive microbial genomics

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Oxford Nanopore continues to support frontline rapid outbreak response

May 2022 - Portugal
First genome of monkeypox from recent multi-country outbreak

Joana Isidro
@isidro_joana

Great team effort and real time data sharing. Overnight sequencing on MinION, raw sequence data available by 11am -> first #monkeypox draft genome and phylogenetics released by midnight. Further sequencing ongoing!

Nextstrain
@nextstrain

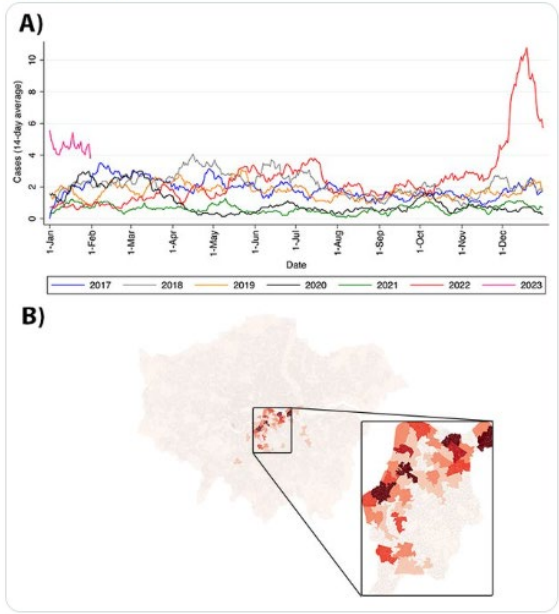
Using #opendata generously shared by @irj_pt and @CDCgov, we have launched nextstrain.org/monkeypox to track genomic epidemiology of the emerging monkeypox virus outbreak. 1/7

Dec. 2022 – United Kingdom
Prospective surveillance of Group A Strep outbreak

Luke Blagdon Snell
@lukebsnell

Today @CMIJournal: Analysis of the Group A Strep epidemic in London. Cases @ 4x usual & invasive disease assoc w/ superantigens A&J doi.org/10.1016/j.cmi....

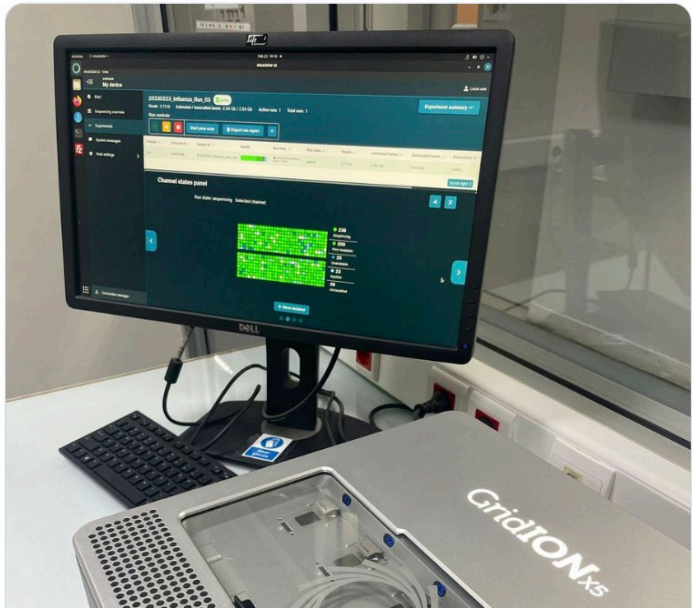
Seq & submitted in 4wks: rapid @nanopore genomics! @madelaam @ThemisCharalam1 @stuartjdneil @GaiaNebbia @jonathanCIDR



Feb, 2023 - Cambodia
Full genome from human infection with avian influenza (H5N1)

Erik Karlsson
@E_A_Karlsson

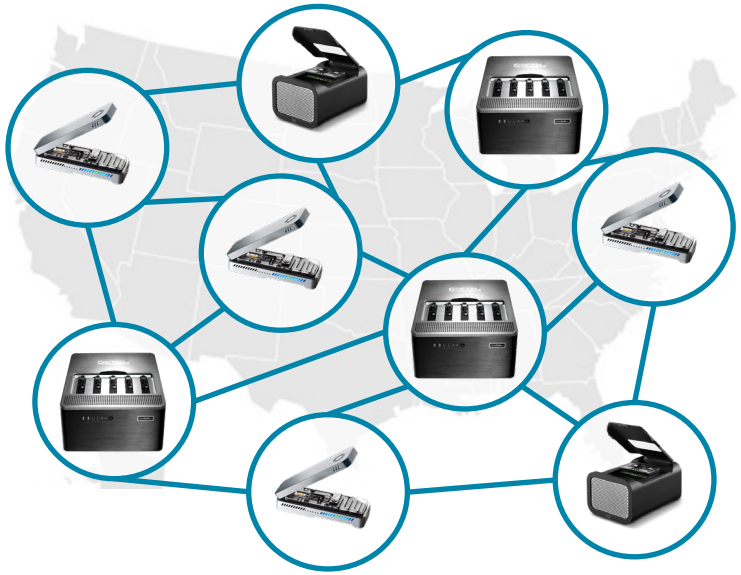
We were able to go from original sample to full genome sequence in <24 hours using iMS-PCR from @peter_thielen from @JHUAPL on @oxfordnanopore #ONT technology @nanopore #nanopore using capacity developed before and during the #COVID19 pandemic (2/)



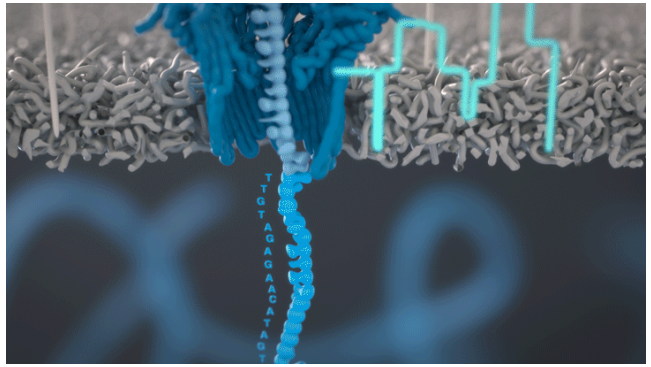
Oxford NANOPORE Technologies

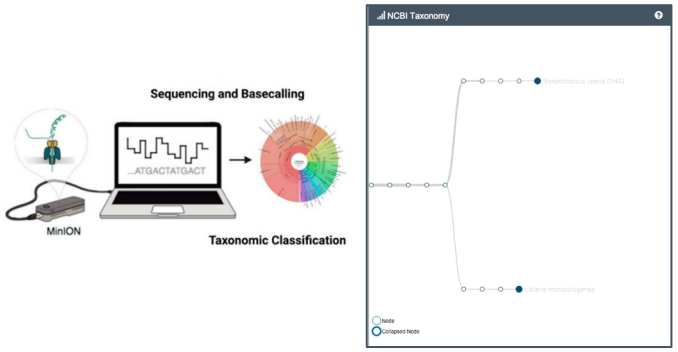
Advantages of implementing Oxford Nanopore Technologies

Accessible platforms empower any lab to deploy genomic sequencing, enabling distributed networks



Streamlined workflows and real-time data deliver rapid turnaround from sample to answer





Long, accurate reads generate complete microbial genomes and resolve plasmids, mobile elements

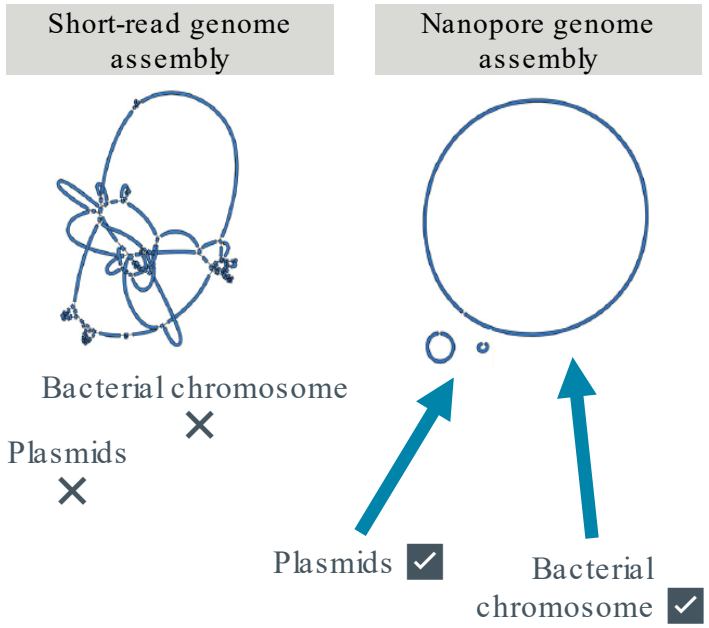
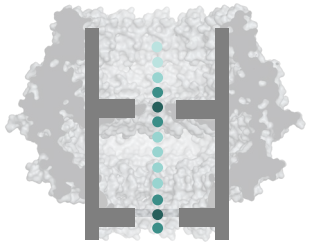


Figure from Ryan Wick, University of Melbourne, Australia

Q20+ chemistry updates deliver high accuracy

Results from the community



Kit V14, R10.4.1

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Biotechnology | Full-Length Text

The newest Oxford Nanopore R10.4.1 full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling

Tianyuan Zhang,[1,2,3] Hanzhou Li,[3] Silin Ma,[1] Jian Cao,[3] Hao Liao,[1] Qiaoyun Huang,[1,4] Wenli Chen[1,2]

Improved Resolution of Highly Pathogenic Avian Influenza Virus Haemagglutinin Cleavage Site Using Oxford Nanopore R10 Sequencing Chemistry

Jeremy D Ratcliff, Brian Merritt, Hannah Gooden, Jurre Y Siegers, Abhi Srikanth, Sokhoun Yann, Sonita Kol, Sarath Sin, Songha Tok, Erik A Karlsson, Peter M Thielen

Do we still need Illumina sequencing data?: Evaluating Oxford Nanopore Technologies R10.4.1 flow cells and v14 library prep kits for Gram negative bacteria whole genome assemblies

Nicole Lerminiaux, Ken Fakharuddin, Michael R. Mulvey, Laura Mataseje

BRIEF REPORT Open Access

Nanopore long-read-only metagenomics enables complete and high-quality genome reconstruction from mock and complex metagenomes

Lei Liu, Yu Yang, Yu Deng and Tong Zhang*

Oxford nanopore long-read sequencing enables the generation of complete bacterial and plasmid genomes without short-read sequencing

Wenxuan Zhao[1,2], Wei Zeng[1,3], Bo Pang[1], Ming Luo[4], Yao Peng[1], Jialiang Xu[5], Biao Kan[1,3]*, Zhenpeng Li[1]* and Xin Lu[1]*

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EPIDEMIOLOGY

Real-Time Nanopore Q20+ Sequencing Enables Extremely Fast and Accurate Core Genome MLST Typing and Democratizes Access to High-Resolution Bacterial Pathogen Surveillance

Gabriel E. Wagner,[a] Johanna Dabernig-Heinz,[a] Michaela Lipp,[a] Adriana Cabal,[b] Jonathan Simantzik,[c] Matthias Kohl,[c] Martina Scheiber,[a] Sabine Lichtenegger,[a] Ralf Ehricht,[d,e,f] Eva Leitner,[a] Werner Ruppitsch,[b] Ivo Steinmetz[a]

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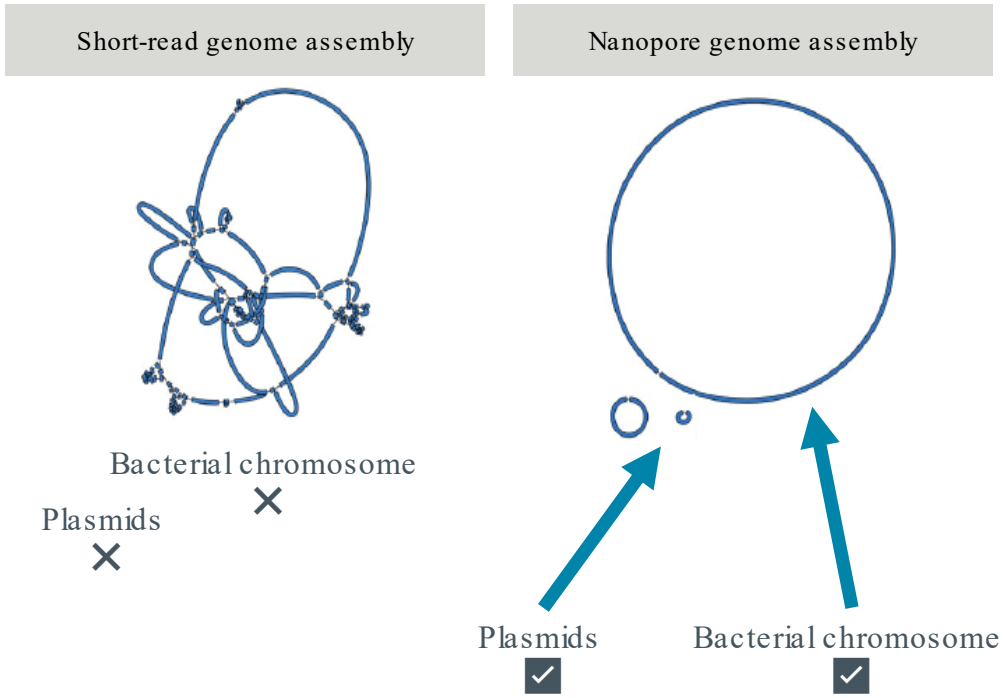
Generating contiguous, high-quality bacterial genome and plasmid assemblies

- Short-read data can struggle to resolve repeat regions larger than the library size, resulting in fragmented, incomplete genome assemblies[1]

- Nanopore sequencing can greatly improve genome assembly, with the ability to
  - Produce complete, closed genome assemblies and resolve genomic context of the chromosome and plasmids
  - Genome contiguity and complete plasmid sequences are particularly important to understand mechanisms and drivers of antimicrobial resistance in bacterial pathogens

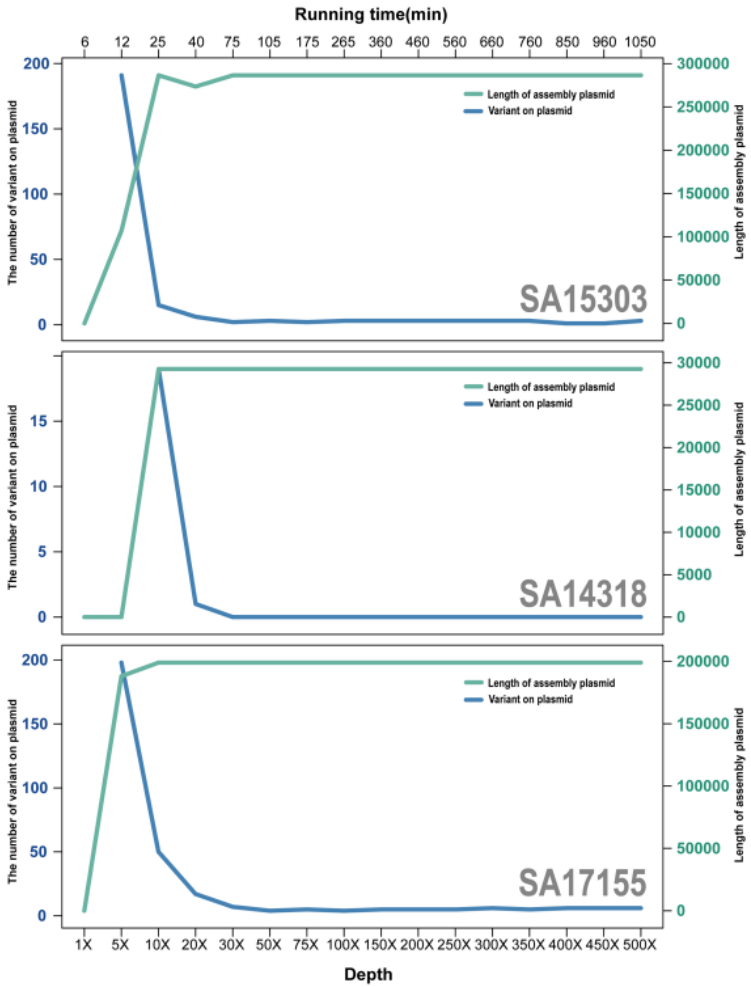


1. Koren and Phillippy. "One chromosome, one contig: complete microbial genomes from long-read sequencing and assembly." Current opinion in microbiology 23 (2015): 110-120.
Figure from Ryan Wick, University of Melbourne, Australia

Case study: complete, high-quality genomes and plasmids from MDR Salmonella

- Authors assessed ONT R10.4.1 chemistry on three strains of Salmonella carrying multidrug resistant (MDR) plasmids
  - Reported average nucleotide identity >99.9975% between each assembled sequence and reference genome
  - Figure (right): trend plot of plasmid size (green) and variants (blue) with increasing sequencing depth

"This method will save time in obtaining high-quality complete genomes of bacteria for antimicrobial resistance surveillance, and is likely to become a valuable tool for monitoring the transmission of plasmid-borne drug resistance genes" – Zhao et al. (2023)

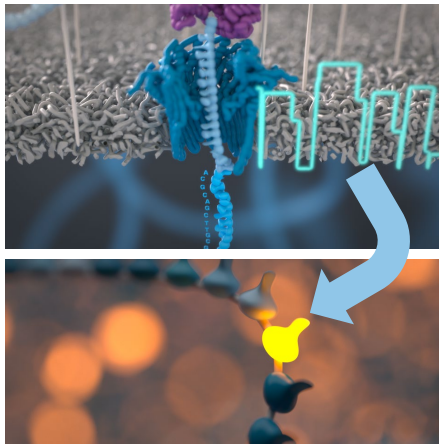


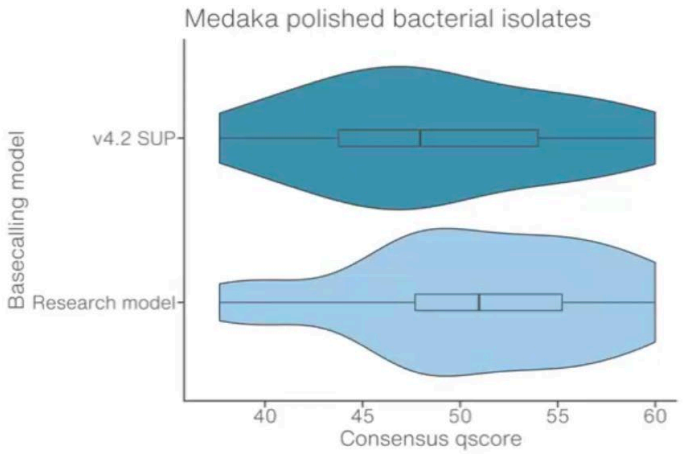
Zhao, et al. "Oxford nanopore long-read sequencing enables the generation of complete bacterial and plasmid genomes without short-read sequencing." Frontiers in Microbiology 14 (2023): 1179966.

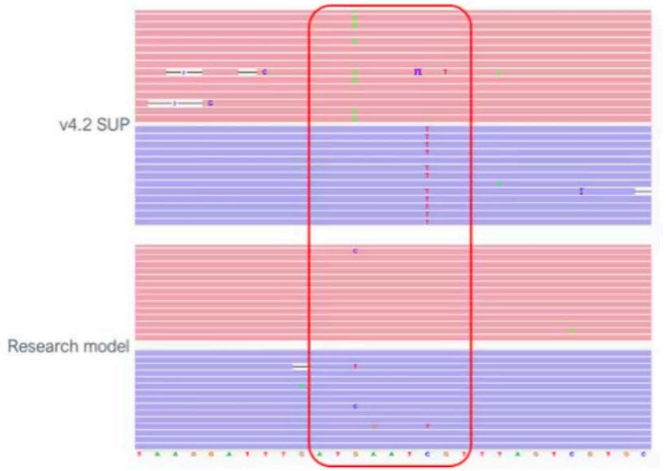
Updates on basecalling model training for bacterial modifications

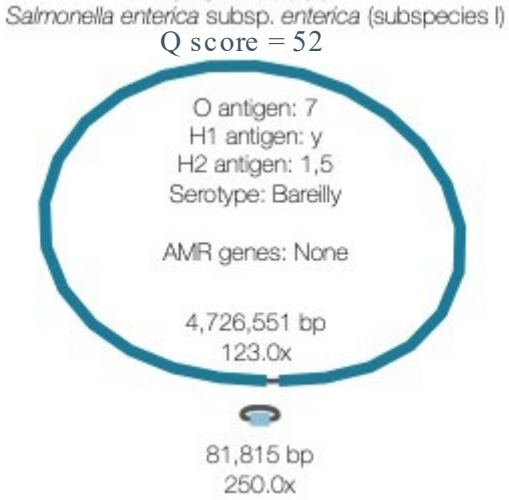
- Certain bacterial genomics DNA samples found to exhibit strain-biased base calling differences which were not present in PCR data
- This is a signature of base modification in the nanopore signal
- These signal perturbations enable mod-calling in Remora (e.g. 5mC, 6mA)
- Research base caller trained on a set of representative samples and spike-in PCR data improves consensus accuracy across the board. Production model in prep

Epigenetic modifications









https://github.com/nanoporetech/rerio

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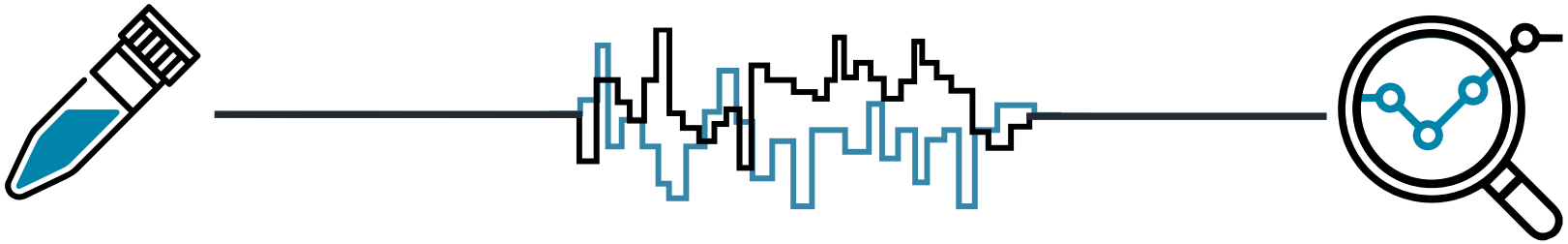
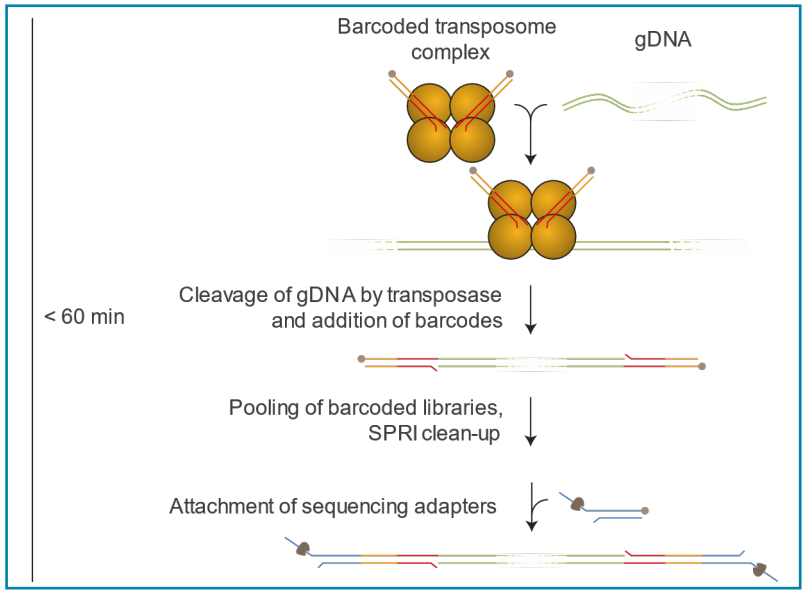
Rapid, complete workflow for bacterial isolate WGS



Prepare

Kit: Rapid Barcoding V14



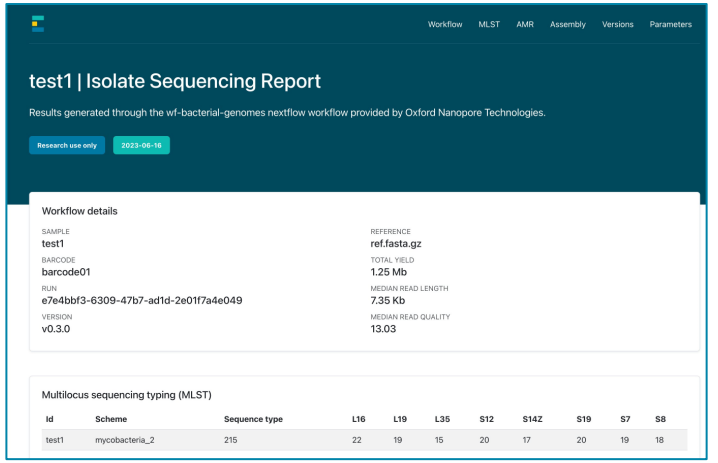
Sequence

Flow cell: R10.4.1

Analyse

Software: https://labs.epi2me.io

wf-bacterial-genomes EPI2ME Labs



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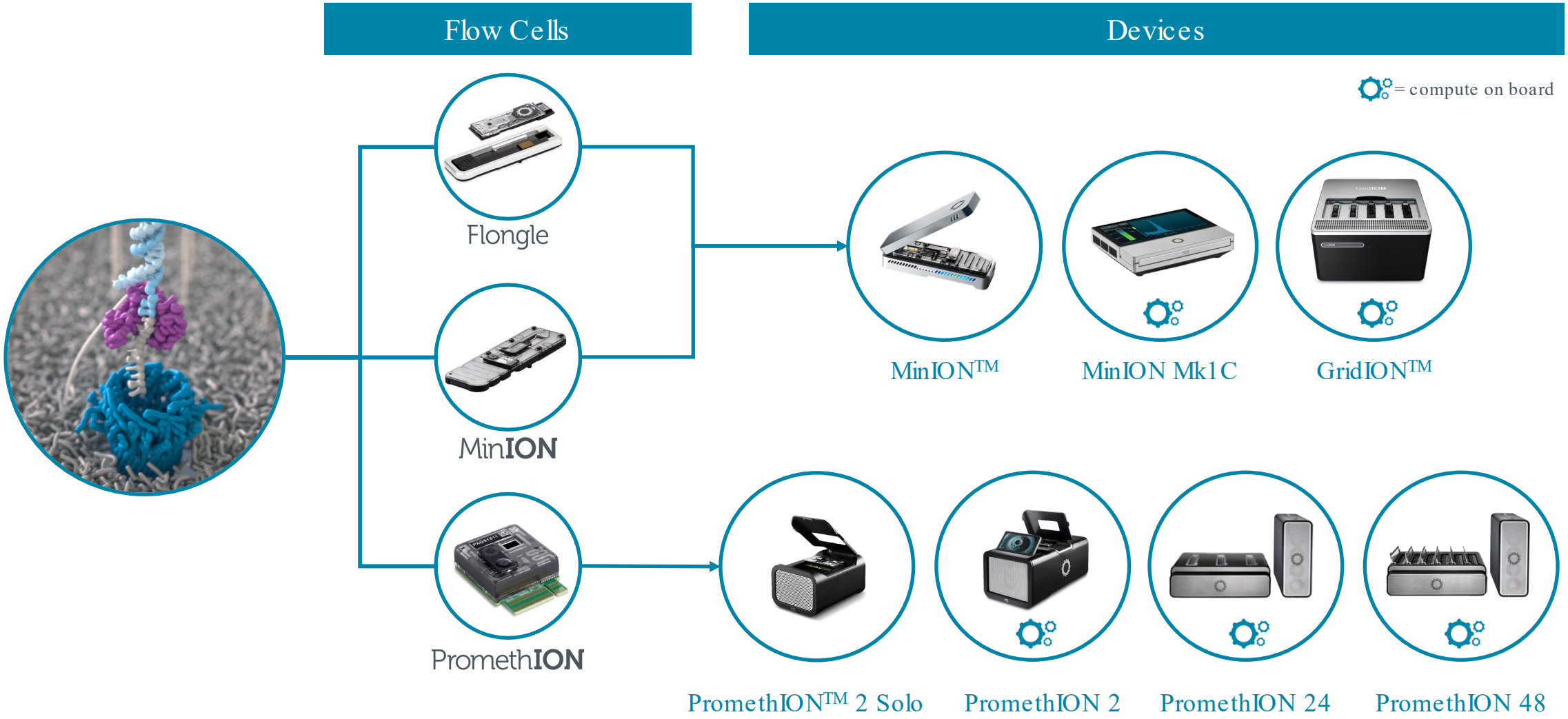
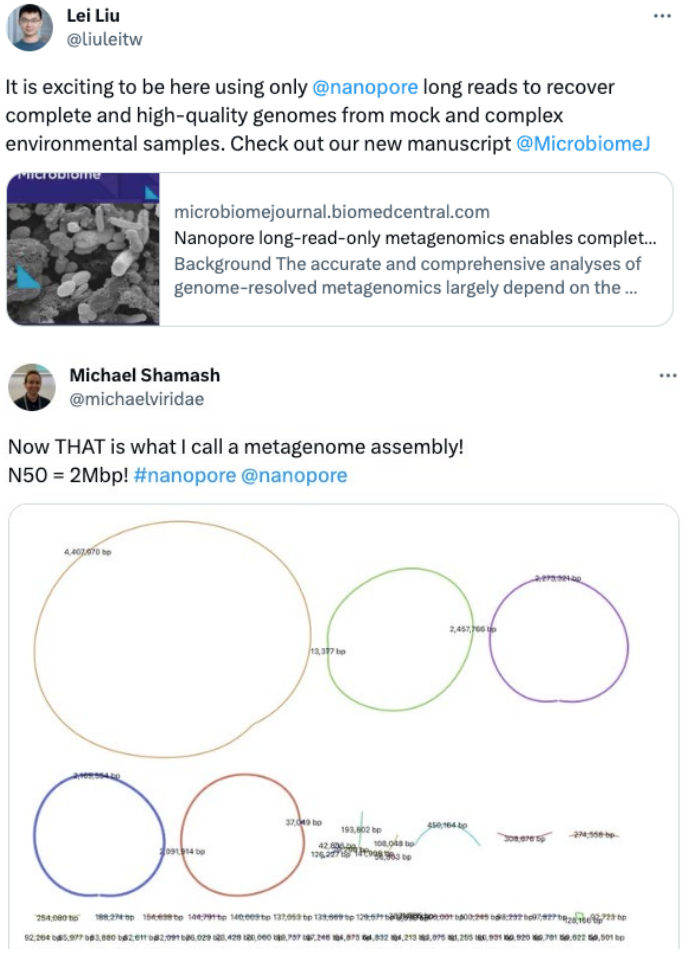
One core technology at any scale

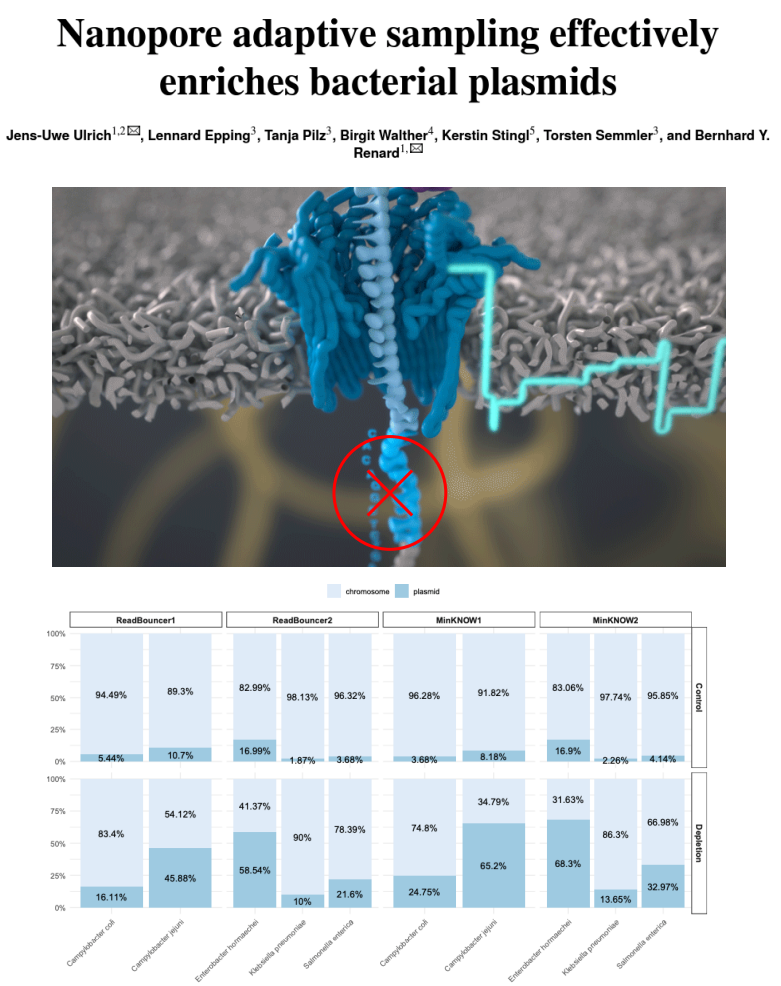


Some exciting applications we didn't get to today

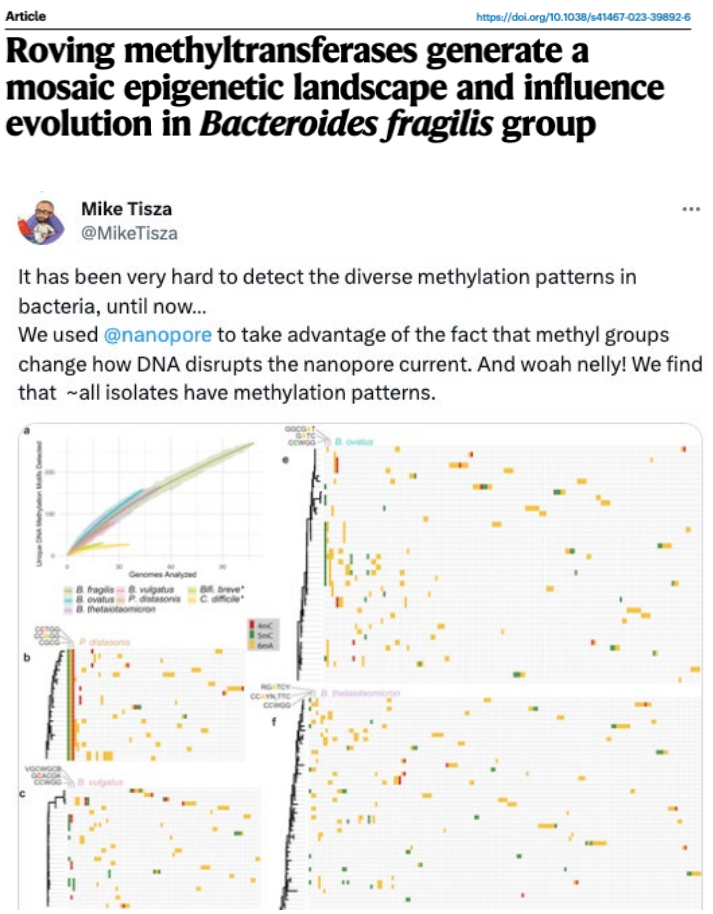
Nanopore-only metagenomics



Adaptive sampling



Bacterial methylation



Thank you

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