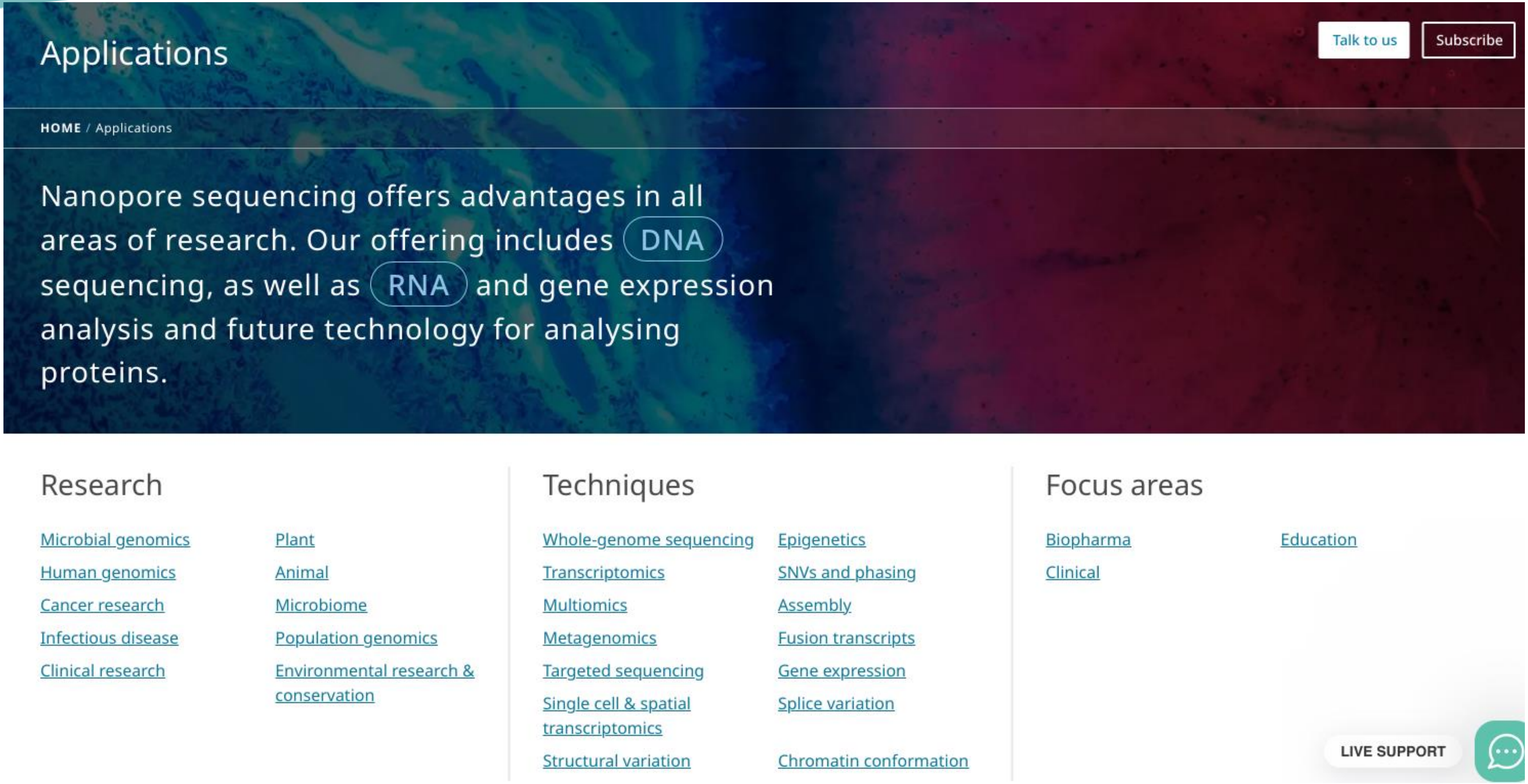
Nanopore Sequencing Technology

State-of-the-art – literature on ONT sequencing applied to food safety, microbiology research and public health surveillance

Niamh Lacy-Roberts, Global Capacity Building Group, DTU, nlac@food.dtu.dk

11th April 2025



Applications

HOME / Applications

Nanopore sequencing offers advantages in all areas of research. Our offering includes DNA sequencing, as well as RNA and gene expression analysis and future technology for analysing proteins.

Research

- Microbial genomics
- Human genomics
- Cancer research
- Infectious disease
- Clinical research
- Plant
- Animal
- Microbiome
- Population genomics
- Environmental research & conservation

Techniques

- Whole-genome sequencing
- Transcriptomics
- Multiomics
- Metagenomics
- Targeted sequencing
- Single cell & spatial transcriptomics
- Structural variation
- Epigenetics
- SNVs and phasing
- Assembly
- Fusion transcripts
- Gene expression
- Splice variation
- Chromatin conformation

Focus areas

- Biopharma
- Clinical
- Education

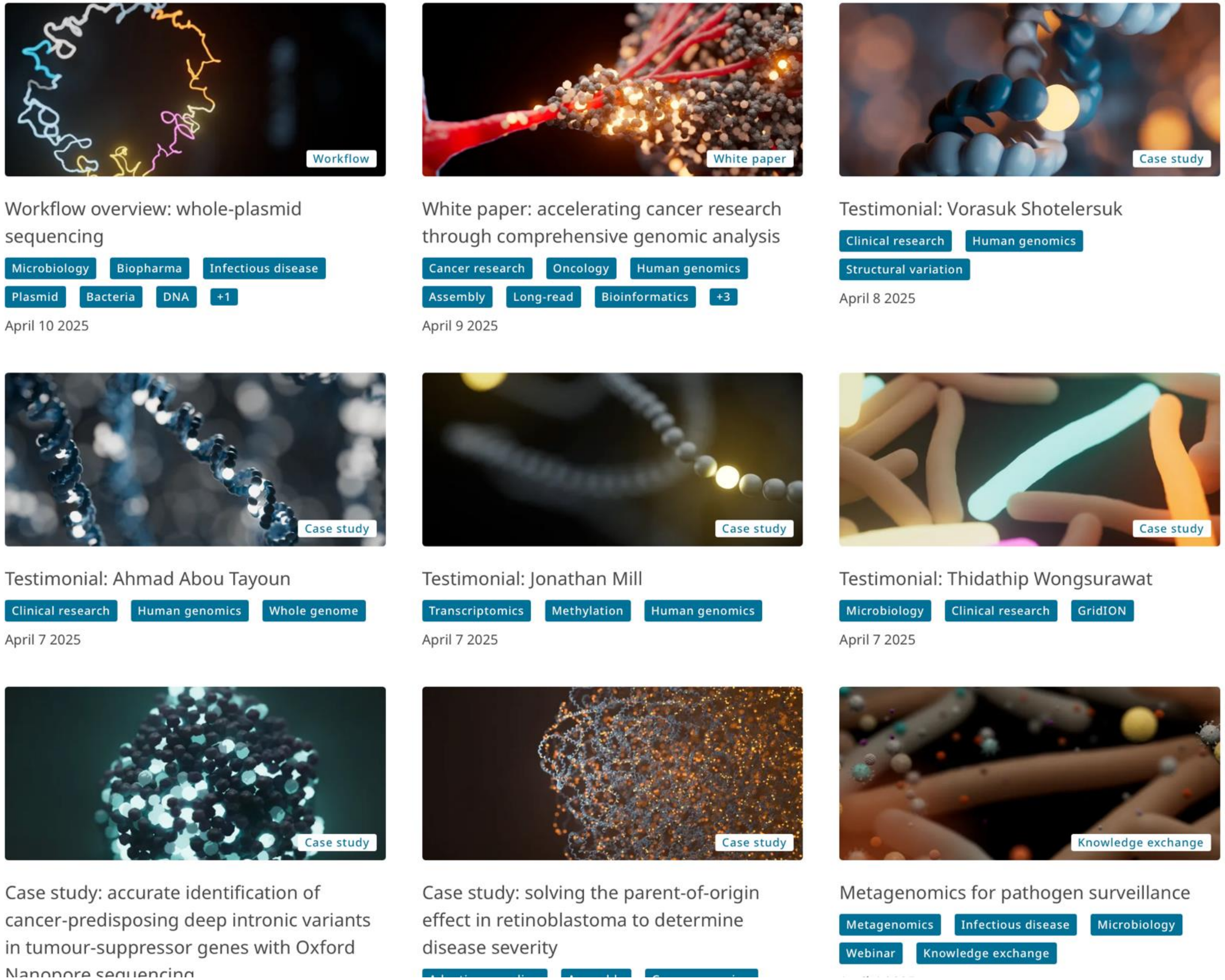
https://nanoporetech.com/applications

Current applications of ONT sequencing

ONT have a lot of resources on their website

- Linking to new original papers
- Nanopore reports (White papers)
- Protocols and workflows
- Webinars

- https://nanoporetech.com/about/events



Workflow overview: whole-plasmid sequencing

Microbiology · Biopharma · Infectious disease · Plasmid · Bacteria · DNA · +1

April 10 2025

White paper: accelerating cancer research through comprehensive genomic analysis

Cancer research · Oncology · Human genomics · Assembly · Long-read · Bioinformatics · +3

April 9 2025

Testimonial: Vorasuk Shotelersuk

Clinical research · Human genomics · Structural variation

April 8 2025

Testimonial: Ahmad Abou Tayoun

Clinical research · Human genomics · Whole genome

April 7 2025

Testimonial: Jonathan Mill

Transcriptomics · Methylation · Human genomics

April 7 2025

Testimonial: Thidathip Wongsurawat

Microbiology · Clinical research · GridION

April 7 2025

Case study: accurate identification of cancer-predisposing deep intronic variants in tumour-suppressor genes with Oxford Nanopore sequencing

Case study: solving the parent-of-origin effect in retinoblastoma to determine disease severity

Metagenomics for pathogen surveillance

Metagenomics · Infectious disease · Microbiology · Webinar · Knowledge exchange

https://nanoporetech.com/resource-centre

Current applications of ONT sequencing

Broad range of applications

- DNA/RNA
- Transcriptomics
- Metagenomics
- Single-cell sequencing
- Single-cell transcriptomics
- ….

Nanopore Tech. have a lot of resources on their website

- Linking to new original papers
- Nanopore reports (White papers)
- Protocols and workflows
- Webinars

NANOPORE

INFECTIOUS DISEASE

Delivering the future of genomic pathogen surveillance with nanopore sequencing

WHITE PAPER

August 2024

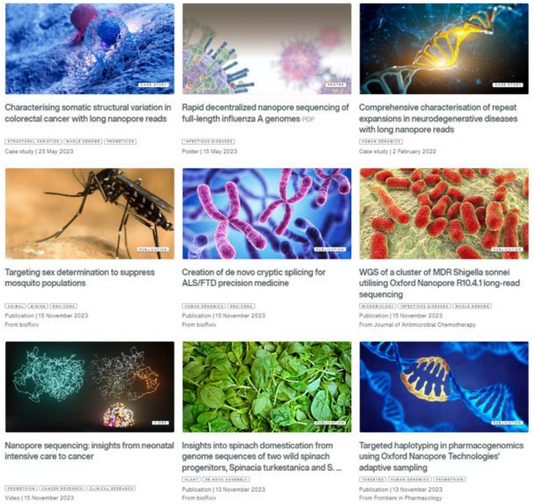
nanoporetech.com

https://nanoporetech.com/resource-centre

Current applications of ONT sequences

Broad range of applications

- Diagnostics
- AMR analysis
- Plasmid analysis
- Outbreak detection



Characterising somatic structural variation in colorectal cancer with long nanopore reads
Case study | 25 May 2023

Rapid decentralized nanopore sequencing of full-length influenza A genomes PDF
Poster | 15 May 2023

Comprehensive characterisation of repeat expansions in neurodegenerative diseases with long nanopore reads
Case study | 2 February 2022

Targeting sex determination to suppress mosquito populations
Publication | 15 November 2023
From bioRxiv

Creation of de novo cryptic splicing for ALS/FTD precision medicine
Publication | 15 November 2023
From bioRxiv

WGS of a cluster of MDR Shigella sonnei utilising Oxford Nanopore R10.4.1 long-read sequencing
Publication | 15 November 2023
From Journal of Antimicrobial Chemotherapy

Nanopore sequencing: insights from neonatal intensive care to cancer
Video | 15 November 2023

Insights into spinach domestication from genome sequences of two wild spinach progenitors, Spinacia turkestanica and S. ...
Publication | 13 November 2023
From bioRxiv

Targeted haplotyping in pharmacogenomics using Oxford Nanopore Technologies' adaptive sampling
Publication | 13 November 2023
From Frontiers in Pharmacology

WGS of a cluster of MDR Shigella sonnei utilising Oxford Nanopore R10.4.1 long-read sequencing
MICROBIOLOGY | INFECTIOUS DISEASES | WHOLE GENOME
Publication | 15 November 2023
From Journal of Antimicrobial Chemotherapy

Routine metagenomics service for intensive care unit patients with respiratory infection
MINION | GRIDION | METAGENOMICS
Publication | 7 November 2023
From American Journal of Respiratory and Critical Care Medicine

Targeted nanopore sequencing for bacterial and viral classification
VIDEO | INFECTIOUS DISEASES | MICROBIOLOGY
Video | 12 October 2023

Application of nanopore sequencing to identify antimicrobial resistance genes, mobile genetic elements and virulence fac...
CLINICAL RESEARCH | MICROBIOLOGY | INFECTIOUS DISEASES
Publication | 26 October 2023
From bioRxiv

https://nanoporetech.com/resource-centre

Diagnostics

Single isolate sequencing

Community sequencing
- Metagenomics
  - Direct specimen testing
  - Wastewater surveillance

ONT White Papers

and publications:

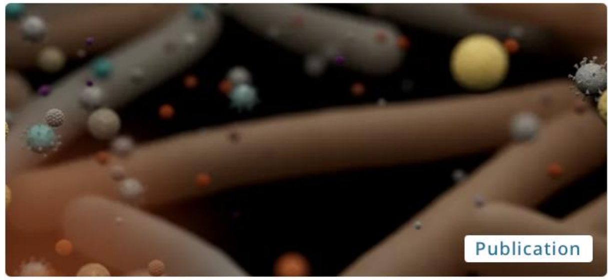
NANOPORE
MICROBIOLOGY
Large insights into microorganisms
WHITE PAPER

NANOPORE
CLINICAL RESEARCH
New approaches for clinical research

Pushing the boundaries of rare disease diagnostics with the help of the first Undiagnosed Hackathon

Human genomics | Whole genome | RNA | DNA

October 21 2024



Real-time application of ITS and D1-D3 nanopore amplicon metagenomic sequencing in fungal infections: enhancing fungal infection diagnostics

Metagenomics | Fungi | Targeted | GridION

July 14 2024

Portable sequencing

Malaria genomic surveillance using nanopore sequencing: Shifting the focus into endemic countries

https://communities.springernature.com/amp/posts/malaria-genomic-surveillance-using-nanopore-sequencing-shifting-the-focus-into-endemic-countries

doi: https://doi.org/10.1101/2022.12.20.521122

The study was based at two sites in Ghana, west Africa, with contrasting epidemiology: the capital, Accra, and Navrongo, a rural town located in northern Ghana.

Real-time DNA barcoding in a rainforest using nanopore sequencing: opportunities for rapid biodiversity assessments and local capacity building

We demonstrate that nanopore sequencing can be implemented in a remote tropical forest to quickly and accurately identify species using DNA barcoding, as we generated consensus sequences for species resolution with an accuracy of >99% in less than 24 hours after collecting specimens.

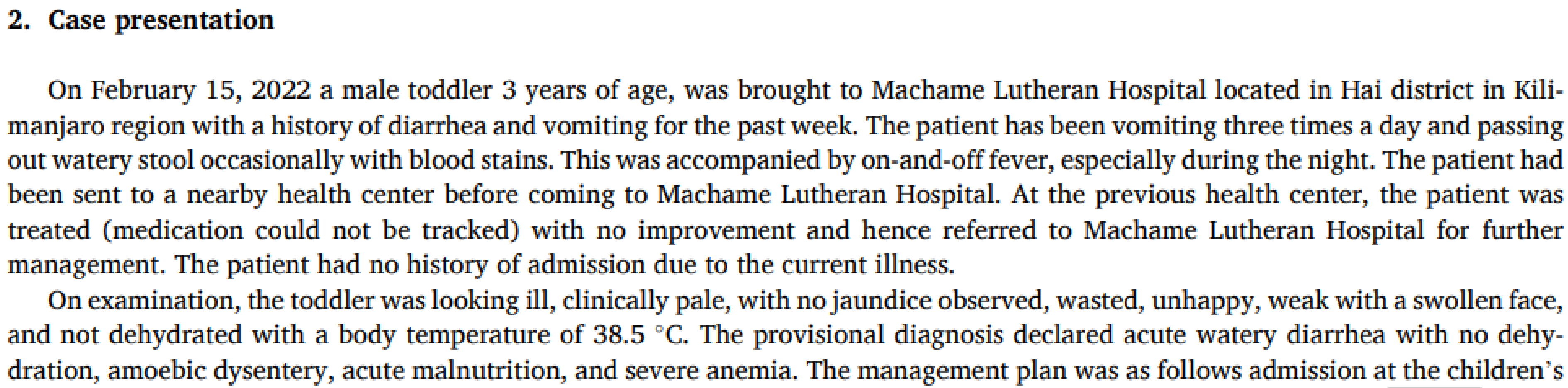
doi: 10.1093/gigascience/giy033

In case of allegations of an intentional cause for a biological threat or disease outbreak, an investigation for verification purposes needs to be able to discriminate between a deliberate release of an agent and the natural background – and rapidly!

INTERNATIONAL
SPACE STATION

To enable the analysis of anything, by anyone, anywhere.

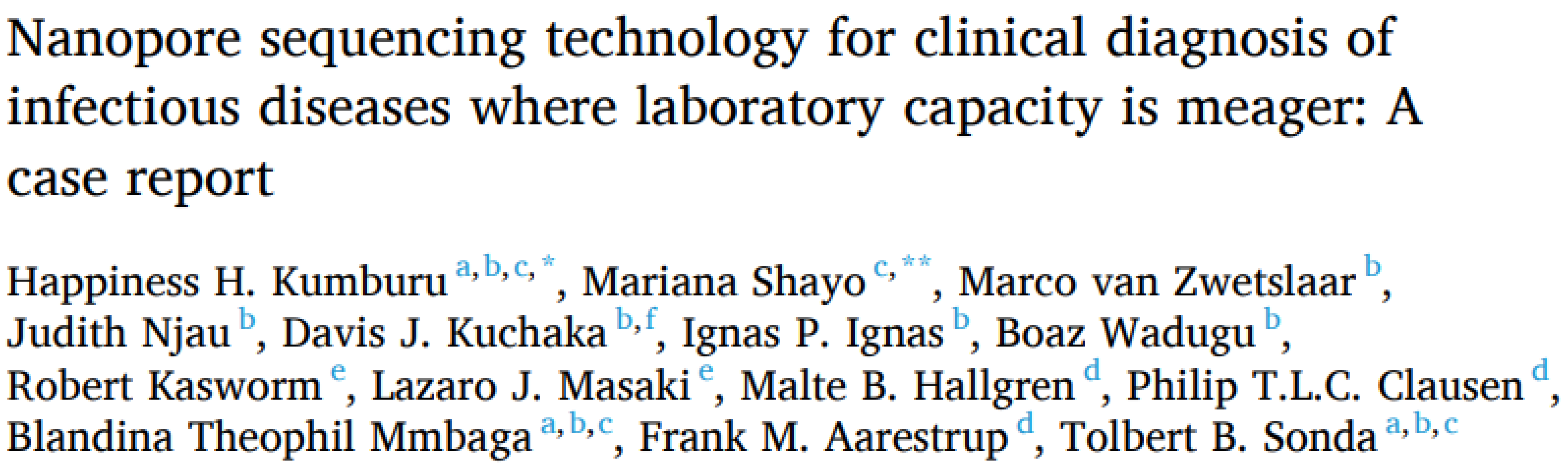
Diagnostics using portable sequencing - example



2. Case presentation

On February 15, 2022 a male toddler 3 years of age, was brought to Machame Lutheran Hospital located in Hai district in Kilimanjaro region with a history of diarrhea and vomiting for the past week. The patient has been vomiting three times a day and passing out watery stool occasionally with blood stains. This was accompanied by on-and-off fever, especially during the night. The patient had been sent to a nearby health center before coming to Machame Lutheran Hospital. At the previous health center, the patient was treated (medication could not be tracked) with no improvement and hence referred to Machame Lutheran Hospital for further management. The patient had no history of admission due to the current illness.

On examination, the toddler was looking ill, clinically pale, with no jaundice observed, wasted, unhappy, weak with a swollen face, and not dehydrated with a body temperature of 38.5 °C. The provisional diagnosis declared acute watery diarrhea with no dehydration, amoebic dysentery, acute malnutrition, and severe anemia. The management plan was as follows admission at the children's



Check for updates

Nanopore sequencing technology for clinical diagnosis of infectious diseases where laboratory capacity is meager: A case report

Happiness H. Kumburu[a,b,c,*], Mariana Shayo[c,**], Marco van Zwetslaar[b],
Judith Njau[b], Davis J. Kuchaka[b,f], Ignas P. Ignas[b], Boaz Wadugu[b],
Robert Kasworm[e], Lazaro J. Masaki[e], Malte B. Hallgren[d], Philip T.L.C. Clausen[d],
Blandina Theophil Mmbaga[a,b,c], Frank M. Aarestrup[d], Tolbert B. Sonda[a,b,c]

https://doi.org/10.1016/j.heliyon.2023.e17439

Diagnostics using portable sequencing – example Faecal microbiota

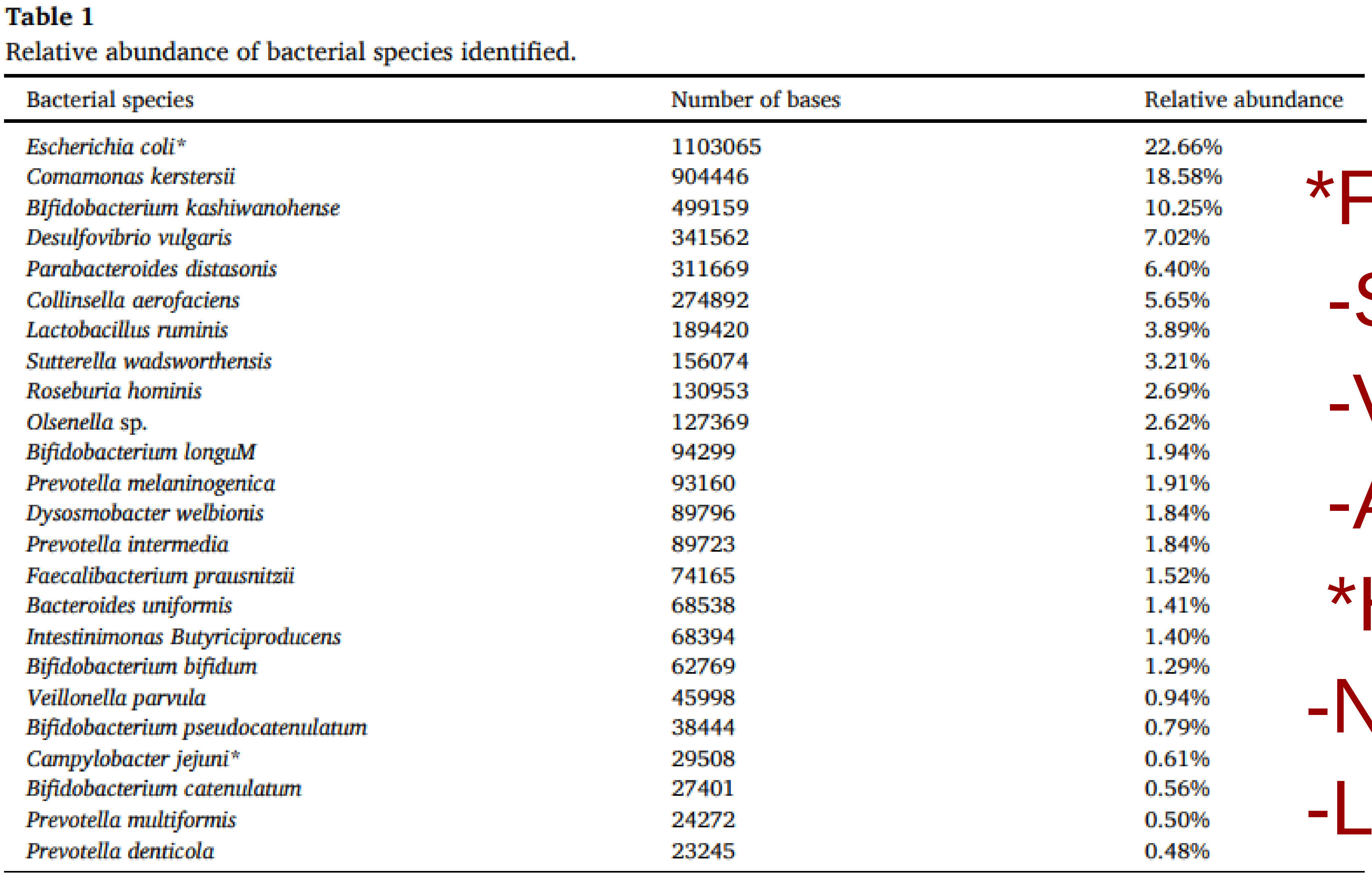


Table 1

Relative abundance of bacterial species identified.

Bacterial species	Number of bases	Relative abundance
*Escherichia coli**	1103065	22.66%
Comamonas kerstersii	904446	18.58%
BIfidobacterium kashiwanohense	499159	10.25%
Desulfovibrio vulgaris	341562	7.02%
Parabacteroides distasonis	311669	6.40%
Collinsella aerofaciens	274892	5.65%
Lactobacillus ruminis	189420	3.89%
Sutterella wadsworthensis	156074	3.21%
Roseburia hominis	130953	2.69%
Olsenella sp.	127369	2.62%
Bifidobacterium longuM	94299	1.94%
Prevotella melaninogenica	93160	1.91%
Dysosmobacter welbionis	89796	1.84%
Prevotella intermedia	89723	1.84%
Faecalibacterium prausnitzii	74165	1.52%
Bacteroides uniformis	68538	1.41%
Intestinimonas Butyriciproducens	68394	1.40%
Bifidobacterium bifidum	62769	1.29%
Veillonella parvula	45998	0.94%
Bifidobacterium pseudocatenulatum	38444	0.79%
*Campylobacter jejuni**	29508	0.61%
Bifidobacterium catenulatum	27401	0.56%
Prevotella multiformis	24272	0.50%
Prevotella denticola	23245	0.48%

*Fast response
 -Species ID
 -Virulence factors
 -AMR genes
 *High resolution!
-Non-culturable
-Low abundance

Metagenomics for diagnostics

Respiratory metagenomics (RMg) holds promise as a first-line diagnostic test for lower respiratory tract infections.

In principle, it rapidly detects all potential pathogens along with antimicrobial resistance determinants and provides sequence typing for infection control or public health actions.

American Journal of Respiratory and Critical Care Medicine

Home > American Journal of Respiratory and Critical Care Medicine > List of Issues > Just Accepted

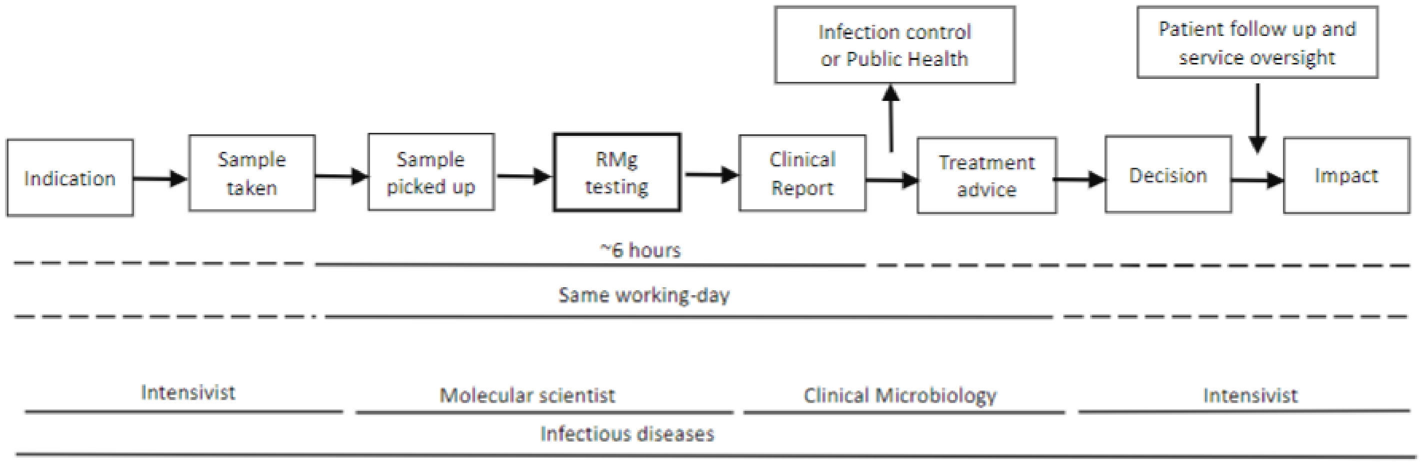
Routine Metagenomics Service for Intensive Care Unit Patients with Respiratory Infection

Themoula Charalampous , Adela Alcolea-Medina , Luke B. Snell , Christopher Alder , Mark Tan , Tom G. S. Williams , Noor Al-Yaakoubi , Gul Humayun , Christopher I S Meadows , Duncan L.A. Wyncoll , Richard Paul , Show All...

Fastidious or unexpected organisms were reported in 21 samples including anaerobes (n=12)
Mycobacterium tuberculosis,
Tropheryma whipplei
Cytomegalovirus
Legionella pneumophila ST1326
Staphylococcus aureus,
Streptococcus pyogenes,
S. dysgalactiae subspecies equisimilis
Aspergillus fumigatus

Metagenomics pilot study

C. Respiratory metagenomics end-to-end clinical pathway



Indication → Sample taken → Sample picked up → RMg testing → Clinical Report → Treatment advice → Decision → Impact

Infection control or Public Health

Patient follow up and service oversight

~6 hours

Same working-day

Intensivist

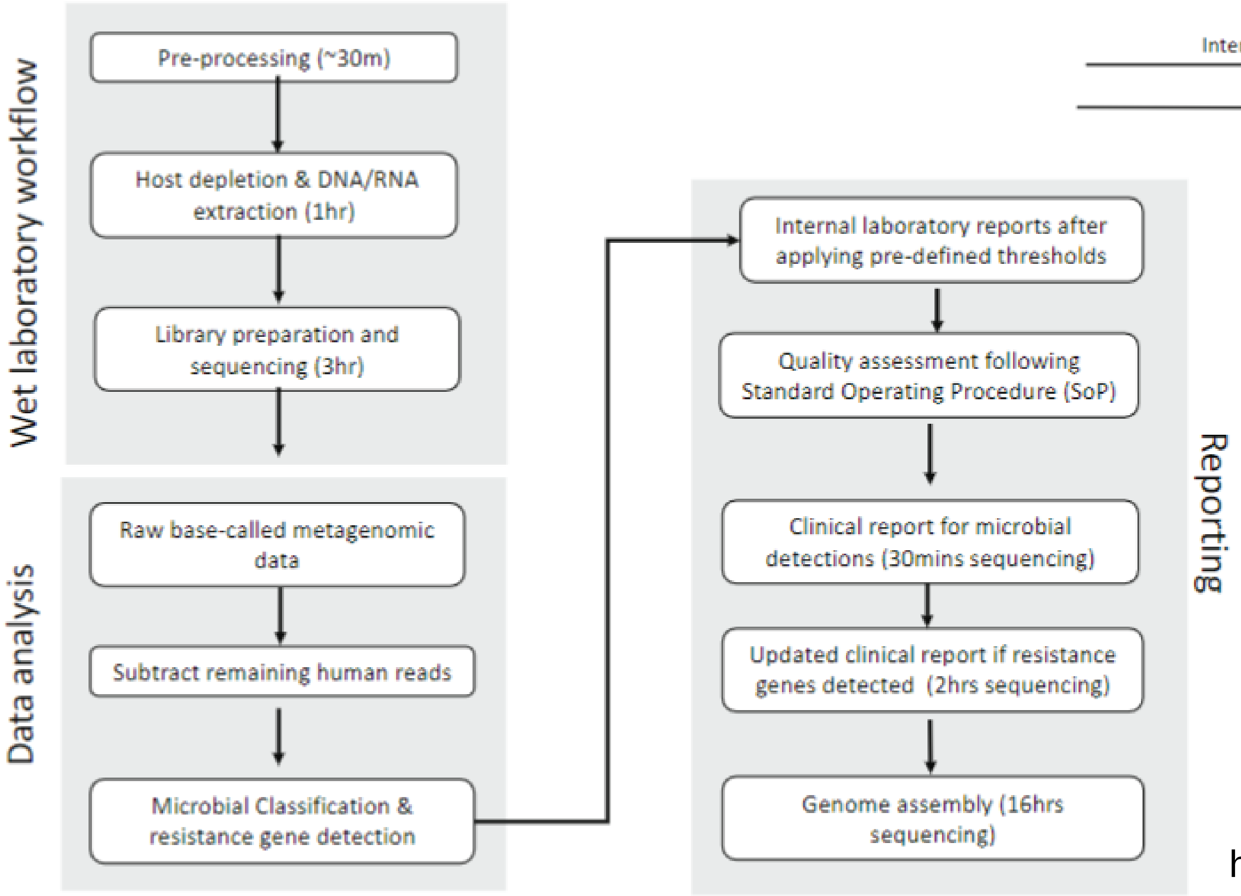
Molecular scientist

Clinical Microbiology

Intensivist

Infectious diseases

B. Respiratory metagenomic Daily Workflow



Wet laboratory workflow

Pre-processing (~30m)

Host depletion & DNA/RNA extraction (1hr)

Library preparation and sequencing (3hr)

Data analysis

Raw base-called metagenomic data

Subtract remaining human reads

Microbial Classification & resistance gene detection

Reporting

Internal laboratory reports after applying pre-defined thresholds

Quality assessment following Standard Operating Procedure (SoP)

Clinical report for microbial detections (30mins sequencing)

Updated clinical report if resistance genes detected (2hrs sequencing)

Genome assembly (16hrs sequencing)

During the first 15-weeks RMg provided same-day results for 110 samples (86%) with median turnaround time of **6.7hrs**

RMg was **93% sensitive** and 81% specific for clinically-relevant pathogens compared with routine testing.

48% of RMg results informed **antimicrobial prescribing changes** (22% escalation; 26% de-escalation) with escalation based on speciation in 20/24 cases and detection of acquired-resistance genes in 4/24 cases

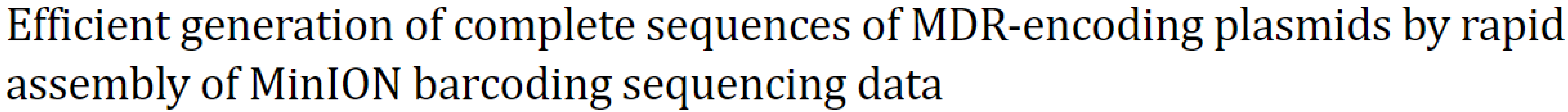
https://doi.org/10.1164/rccm.202305-0901OC

AMR analysis - plasmids

Advantages
- Fast answer
  - Real-time sequencing
- Full genome assembly
- Full plasmid assembly

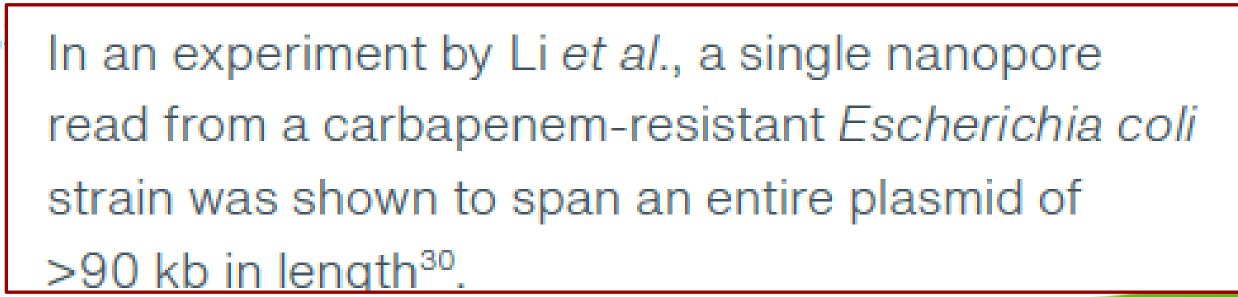
Application of nanopore sequencing to identify antimicrobial resistance genes, mobile genetic elements and virulence factors in clinical isolates

Rachel Kimani, Sebastian Musundi, Patrick Wakaba, David Mbogo, Suliman Essuman, Bernard N. Kanoi, Jesse Gitaka



Efficient generation of complete sequences of MDR-encoding plasmids by rapid assembly of MinION barcoding sequencing data

Ruichao Li,[1,2] Miaomiao Xie,[1] Ning Dong,[1] Dachuan Lin,[1]
Edward Wai-Chi Chan,[2] and Sheng Chen[1,2]



In an experiment by Li *et al.*, a single nanopore read from a carbapenem-resistant *Escherichia coli* strain was shown to span an entire plasmid of >90 kb in length[30].

https://doi.org/10.1101/2023.10.25.563907

doi: 10.1093/gigascience/gix132

Plasmid analysis – ONT only

Before – Illumina/Sanger sequencing

- high-quality plasmid sequence is achieved through capillary-based sequencing, requiring customized sets of primers for each plasmid

Complete sequence verification of plasmid DNA using the Oxford Nanopore Technologies' MinION device

Scott D. Brown, Lisa Dreolini, Jessica F. Wilson, Miruna Balasundaram & Robert A. Holt

BMC Bioinformatics **24**, Article number: 116 (2023) | Cite this article

https://doi.org/10.1186/s12859-023-05226-y

More accurate assemblies

Hybrid assemblies using both short-read and long-read sequences

- Using short reads with high accurracy and long reads with long contigs to improve assemblies
  - E.g. short-read- first assembler, Unicycler

- Using the long contigs of ONT and then do proof-reading with Illumina (or other short read technology)

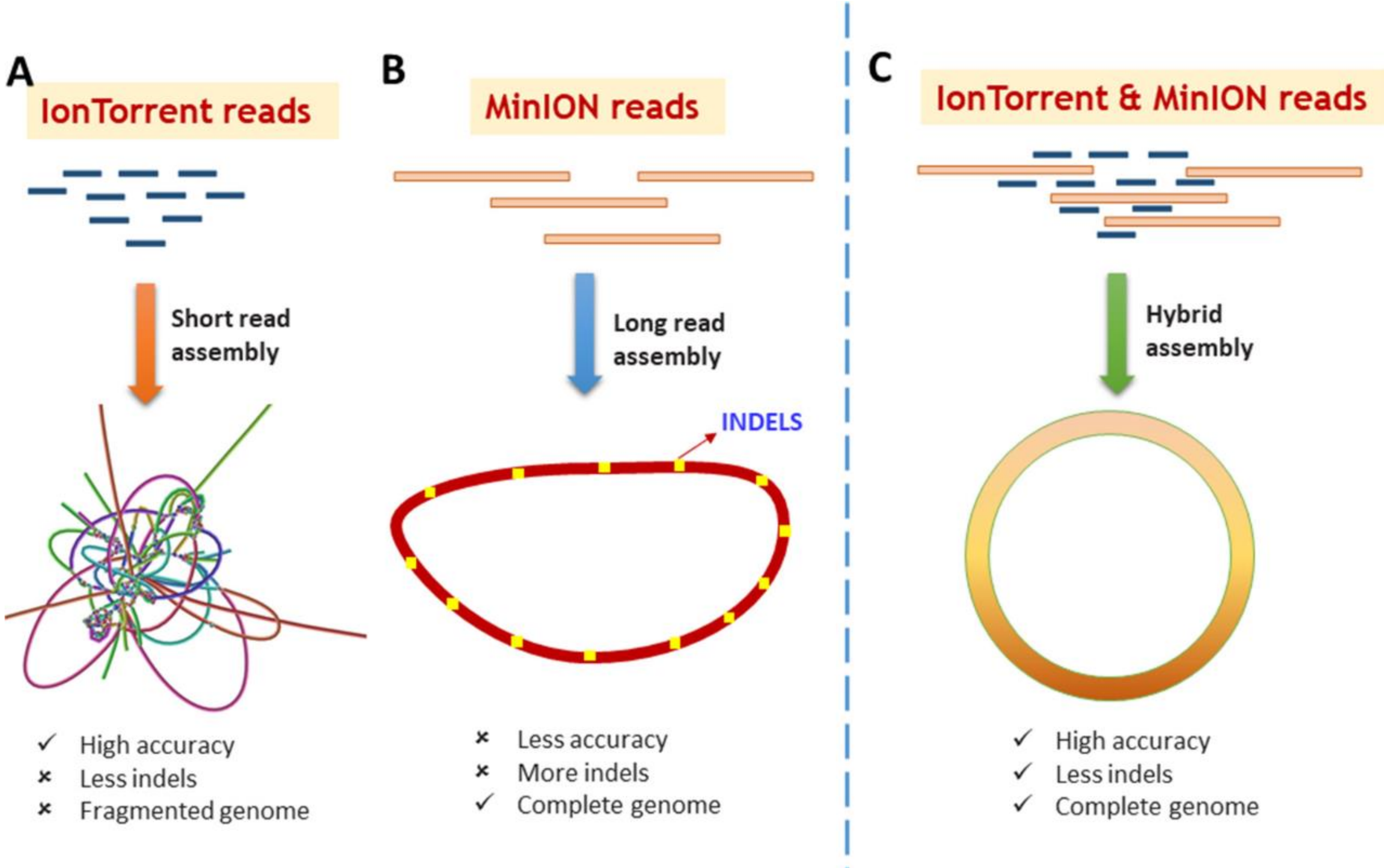
- Using long reads only

Plasmid hybrid assembly

Highly accurate-single chromosomal complete genomes using IonTorrent and MinION sequencing of clinical pathogens

Karthick Vasudevan [1], Naveen Kumar Devanga Ragupathi [1], Jobin John Jacob,
Balaji Veeraraghavan

Requires both short-read and long-read sequences



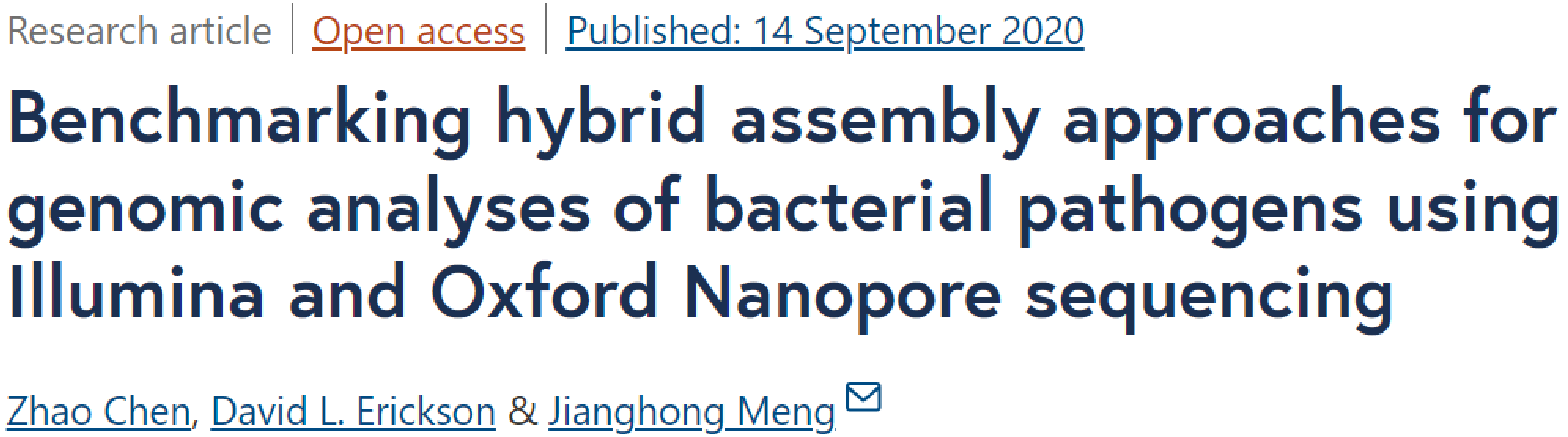
https://doi.org/10.1016/j.ygeno.2019.04.006

Plasmid hybrid assembly approaches

We **benchmarked** the hybrid assembly approaches of

MaSuRCA, SPAdes, and Unicycler

for bacterial pathogens using Illumina and Oxford Nanopore sequencing by determining genome completeness and accuracy, antimicrobial resistance (AMR), virulence potential, multilocus sequence typing (MLST), phylogeny, and pan genome



Research article | Open access | Published: 14 September 2020

Benchmarking hybrid assembly approaches for genomic analyses of bacterial pathogens using Illumina and Oxford Nanopore sequencing

Zhao Chen, David L. Erickson & Jianghong Meng

https://doi.org/10.1186/s12864-020-07041-8

Comparison of ONT library preps

Oxford Nanopore Technologies (ONT) sequencing platforms currently offer two approaches to whole-genome native-DNA library preparation: **ligation and rapid**.

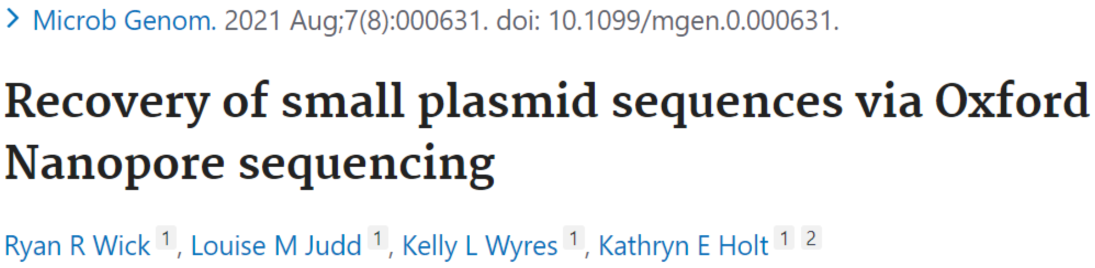
Comparison of these two approaches for assessing their ability to recover small plasmid sequences.

DNA sequenced from seven plasmid-rich bacterial isolates in three different ways:

- ONT ligation, ONT rapid and Illumina.

Using the Illumina read depths to approximate true plasmid abundance

- small plasmids (<20 kbp) were underrepresented in ONT ligation read sets but were not underrepresented in ONT rapid read sets.



> Microb Genom. 2021 Aug;7(8):000631. doi: 10.1099/mgen.0.000631.

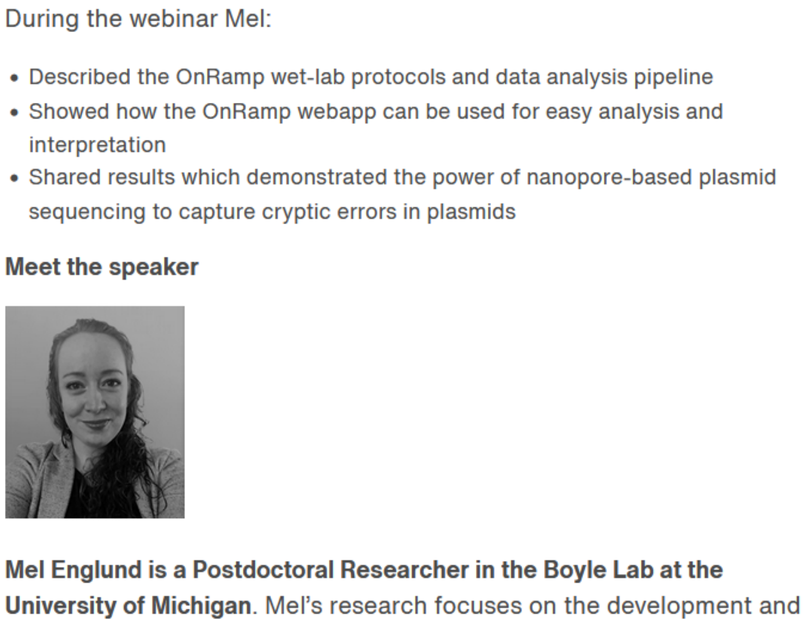
Recovery of small plasmid sequences via Oxford Nanopore sequencing

Ryan R Wick [1], Louise M Judd [1], Kelly L Wyres [1], Kathryn E Holt [1] [2]

- Smallest plasmids being the most underrepresented in ONT ligation read sets.
  - Lower rates of chimaeric reads in the rapid read sets relative to ligation read sets.
  - When small plasmid recovery is important, ONT rapid library preparations are preferable to ligation-based protocols

DOI: 10.1099/mgen.0.000631

Plasmids, plasmids, plasmids…

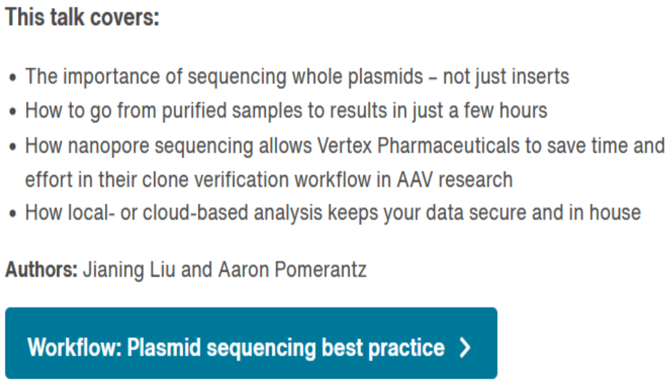


During the webinar Mel:

- Described the OnRamp wet-lab protocols and data analysis pipeline
- Showed how the OnRamp webapp can be used for easy analysis and interpretation
- Shared results which demonstrated the power of nanopore-based plasmid sequencing to capture cryptic errors in plasmids

Meet the speaker

Mel Englund is a Postdoctoral Researcher in the Boyle Lab at the University of Michigan. Mel's research focuses on the development and



This talk covers:

- The importance of sequencing whole plasmids – not just inserts
- How to go from purified samples to results in just a few hours
- How nanopore sequencing allows Vertex Pharmaceuticals to save time and effort in their clone verification workflow in AAV research
- How local- or cloud-based analysis keeps your data secure and in house

Authors: Jianing Liu and Aaron Pomerantz

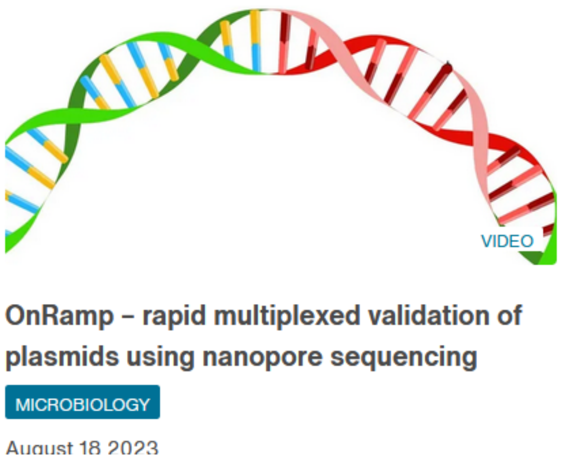
WEBINAR

Core lab webinar series: A new 'gold standard' solution for complete plasmid sequencing

Jemma Jordan, Oxford Nanopore Technologies
Stephen Rudd, Oxford Nanopore Technologies
Mel Englund, University of Michigan

27th July | 3pm (BST)

nanoporetech.com/events



OnRamp – rapid multiplexed validation of plasmids using nanopore sequencing

August 18 2023

Same-day whole-plasmid sequencing in your lab

September 8 2022

Core lab webinar series: A new 'gold standard' solution for complete plasmid sequencing

July 28 2023

Outbreak investigation with ONT

ONT applied to

- produce complete reference sequences
- cluster detection

> To utilize long-read nanopore sequencing (R10.4.1 flowcells) for WGS of a cluster of MDR *Shigella sonnei*, specifically characterizing genetic predictors of antimicrobial resistance

WGS of a cluster of MDR *Shigella sonnei* utilizing Oxford Nanopore R10.4.1 long-read sequencing

Gordon Ritchie ✉, Samuel D Chorlton, Nancy Matic, Jennifer Bilawka, Leah Gowland, Victor Leung, Aleksandra Stefanovic, Marc G Romney, Christopher F Lowe

https://doi.org/10.1093/jac/dkad346

Oxford NANOPORE Technologies

INFECTIOUS DISEASE

Delivering the future of genomic pathogen surveillance with nanopore sequencing

WHITE PAPER

August 2024

nanoporetech.com/resource-centre

nanoporetech.com

Outbreak investigation with ONT

- Detection of AMR genes AND chromosomal mutations
- Cluster isolates were resistant to:
- ampicillin (*bla*$_{\text{TEM-1}}$)
- trimethoprim/sulfamethoxazole (*dfA1*, *dfrA17*; *sul1*, *sul2*),
- azithromycin (*ermB*, *mphA*)
- ciprofloxacin (*gyrA* S83L, *gyrA* D87G, *parC* S80I)

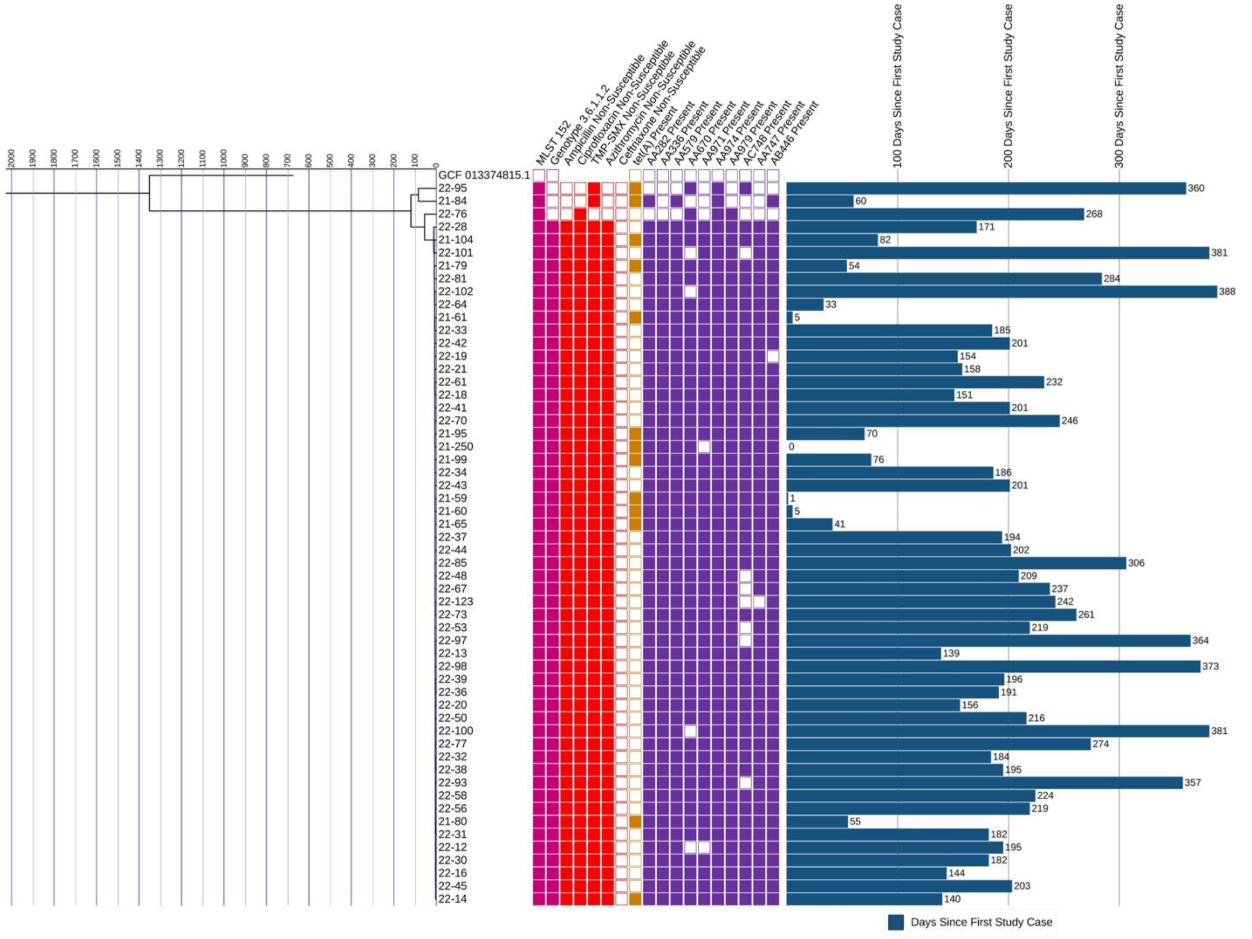


Figure 1. Phylogenetic tree of Shigella sonnei isolated in our laboratory during the study period, including genomic ...

J Antimicrob Chemother, dkad346, https://doi.org/10.1093/jac/dkad346

Salmonella serotyping from colony in 10-20 h

Direct and fast serotyping:

- No DNA extraction
- Multiplex up to 24 samples
- EPI2ME™ analysis integrated with SeqSero2

https://a.storyblok.com/f/196663/x/2df59a9d4c/overview-direct-from-colony-microbial-sequencing-salmonella.pdf

Kits, devices, and software

Library preparation

Rapid PCR Barcoding Kit V14

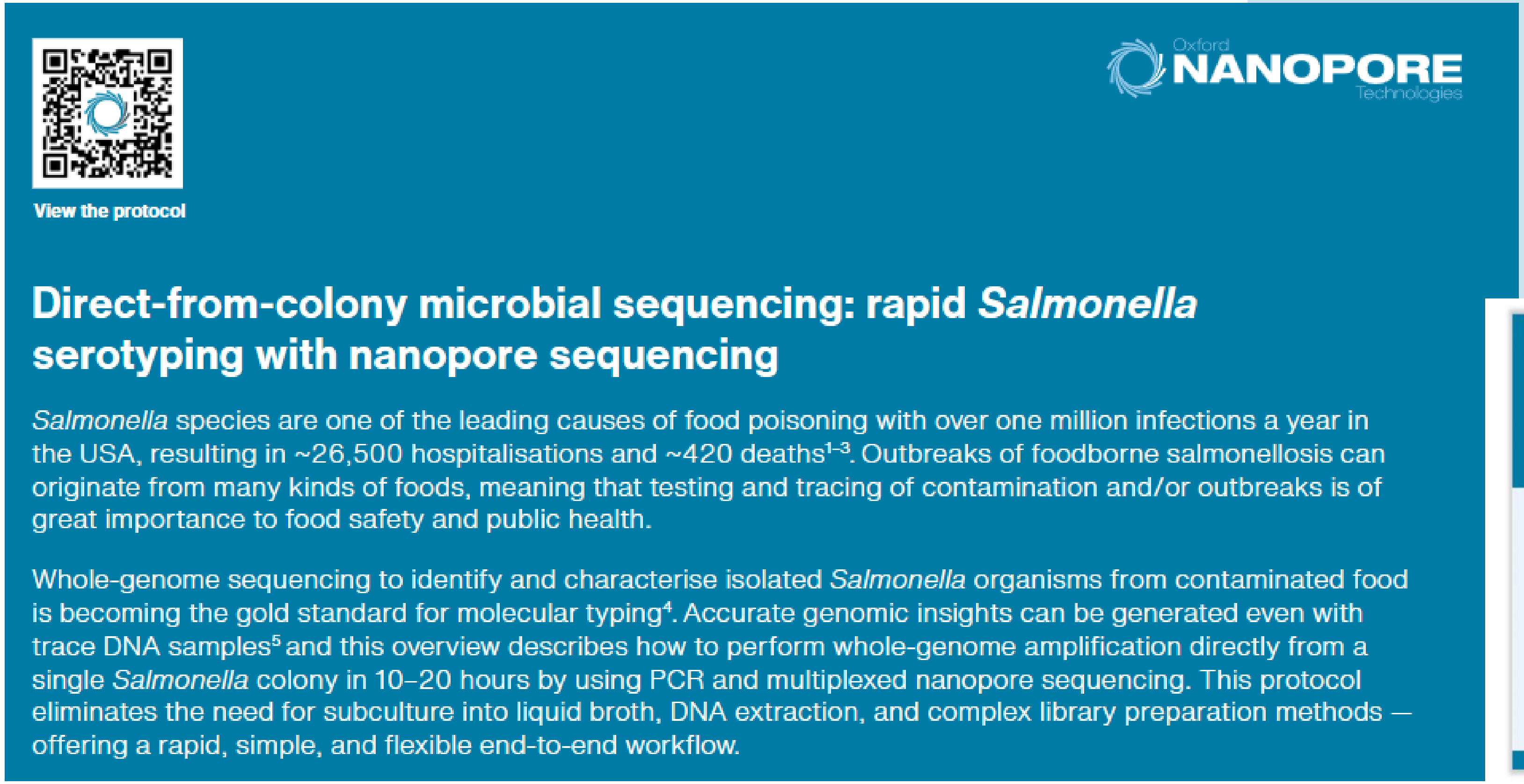
Sequencing

MinION Flow Cells on MinION or GridION devices

Analysis

EPI2ME

labs.epi2me.io



View the protocol

Direct-from-colony microbial sequencing: rapid *Salmonella* serotyping with nanopore sequencing

Salmonella species are one of the leading causes of food poisoning with over one million infections a year in the USA, resulting in ~26,500 hospitalisations and ~420 deaths[1–3]. Outbreaks of foodborne salmonellosis can originate from many kinds of foods, meaning that testing and tracing of contamination and/or outbreaks is of great importance to food safety and public health.

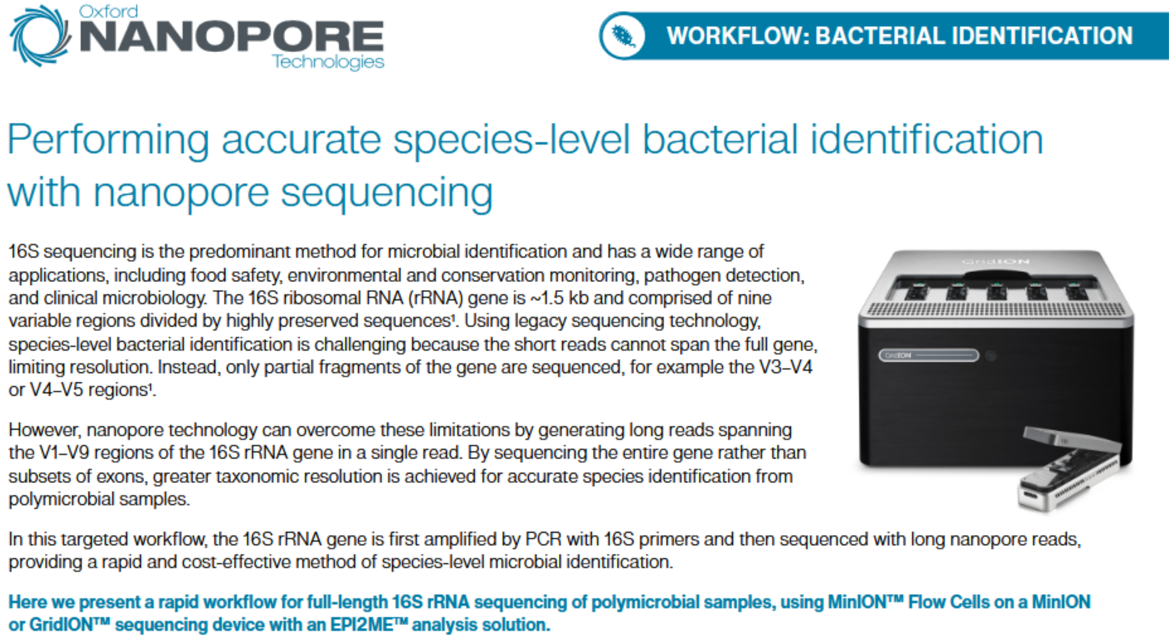
Whole-genome sequencing to identify and characterise isolated *Salmonella* organisms from contaminated food is becoming the gold standard for molecular typing[4]. Accurate genomic insights can be generated even with trace DNA samples[5] and this overview describes how to perform whole-genome amplification directly from a single *Salmonella* colony in 10–20 hours by using PCR and multiplexed nanopore sequencing. This protocol eliminates the need for subculture into liquid broth, DNA extraction, and complex library preparation methods — offering a rapid, simple, and flexible end-to-end workflow.

Polyspecies and quasimetagenomics

Polyspecies example:

16S rRNA profiling for species ID also from complex samples
- Faecal/Caecal
- Food
- Soil/environment

Quasimetagenomics
- (Semi-)selective enrichment and targeted immunomagnetic separation beads
  - Genome assemblies
    - Also applied for e.g. wash water from vegetables


Oxford NANOPORE Technologies

WORKFLOW: BACTERIAL IDENTIFICATION

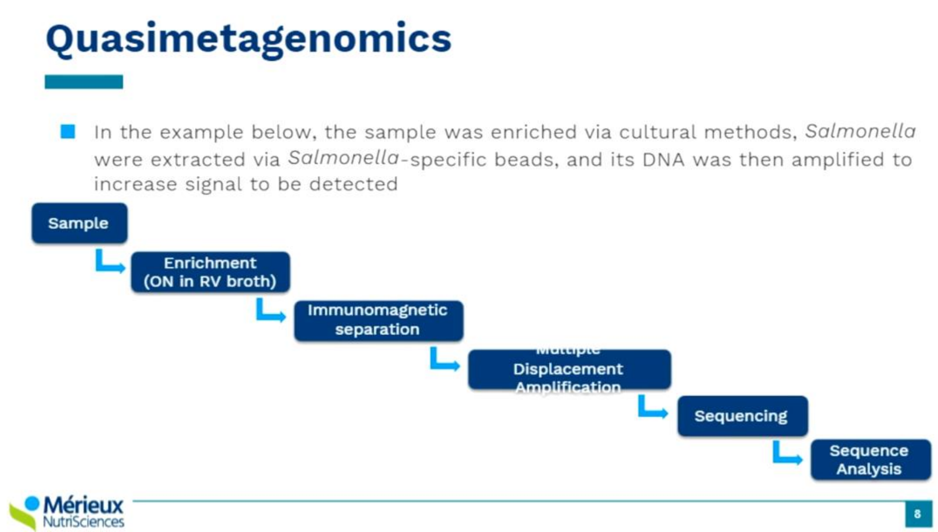
Performing accurate species-level bacterial identification with nanopore sequencing

16S sequencing is the predominant method for microbial identification and has a wide range of applications, including food safety, environmental and conservation monitoring, pathogen detection, and clinical microbiology. The 16S ribosomal RNA (rRNA) gene is ~1.5 kb and comprised of nine variable regions divided by highly preserved sequences[1]. Using legacy sequencing technology, species-level bacterial identification is challenging because the short reads cannot span the full gene, limiting resolution. Instead, only partial fragments of the gene are sequenced, for example the V3–V4 or V4–V5 regions[1].

However, nanopore technology can overcome these limitations by generating long reads spanning the V1–V9 regions of the 16S rRNA gene in a single read. By sequencing the entire gene rather than subsets of exons, greater taxonomic resolution is achieved for accurate species identification from polymicrobial samples.

In this targeted workflow, the 16S rRNA gene is first amplified by PCR with 16S primers and then sequenced with long nanopore reads, providing a rapid and cost-effective method of species-level microbial identification.

Here we present a rapid workflow for full-length 16S rRNA sequencing of polymicrobial samples, using MinION™ Flow Cells on a MinION or GridION™ sequencing device with an EPI2ME™ analysis solution.


Quasimetagenomics

- In the example below, the sample was enriched via cultural methods, *Salmonella* were extracted via *Salmonella*-specific beads, and its DNA was then amplified to increase signal to be detected

Sample → Enrichment (ON in RV broth) → Immunomagnetic separation → Multiple Displacement Amplification → Sequencing → Sequence Analysis

Polyspecies/community and method optimization

Testing different library preps, primers (16S rRNA) and base calling

Moving Beyond Oxford Nanopore Standard Procedures: New Insights from Water and Multiple Fish Microbiomes

by Ricardo Domingo-Bretón [1], Federico Moroni [1], Socorro Toxqui-Rodríguez [1,2], Álvaro Belenguer [1], M. Carla Piazzon [2], Jaume Pérez-Sánchez [1,*] and Fernando Naya-Català [1,*]

https://doi.org/10.3390/ijms252312603

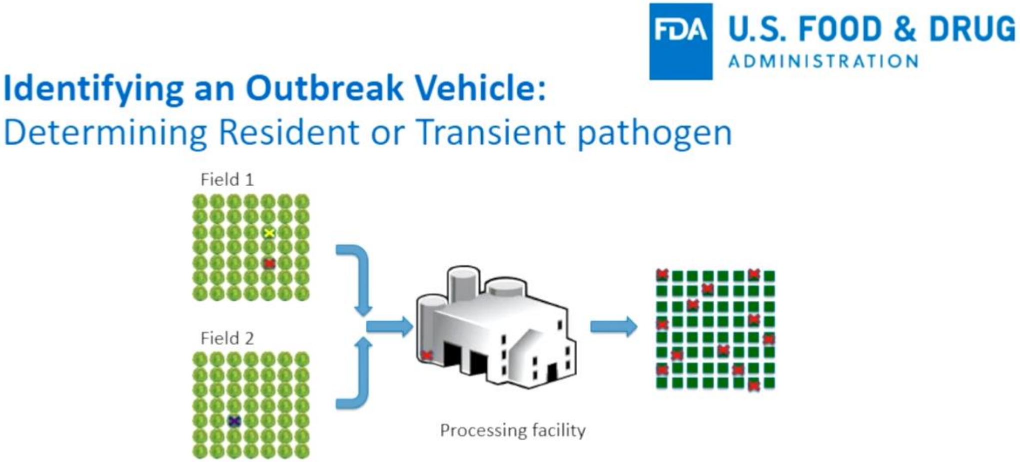
Fast genomic ID and typing

Avoid traditional selctive enrichment, isolation and typing

Helpful for farms and food industry to

- Confirm presence/absence of specific bacteria
- find re-occuring problems
- Measure the effects of desinfection and interventions

Saving time and money!!



U.S. FOOD & DRUG ADMINISTRATION

Identifying an Outbreak Vehicle:
Determining Resident or Transient pathogen

https://nanoporetech.com/resource-centre/advancements-in-nanopore-sequencing-for-enhanced-food-safety-and-quality-assurance

Cultural enrichment adds a bias

Using selective enrichment from food or faecal samples

- Typically one isolate per sample
- Risk of overlooking important isolates

- Adaptive sampling and shotgun sequencing

- No need for culture enrichment, enabling faster investigations and facilitating precise pathogen characterization
- More efficient and targeted analysis of microbial communities

frontiers | Frontiers in Microbiology

Strain-level characterization of foodborne pathogens without culture enrichment for outbreak investigation using shotgun metagenomics facilitated with nanopore adaptive sampling

Florence E. Buytaers[1,2], Bavo Verhaegen[3], Tom Van Nieuwenhuysen[3], Nancy H. C. Roosens[1], Kevin Vanneste[1], Kathleen Marchal[2,4] and Sigrid C. J. De Keersmaecker[1]*

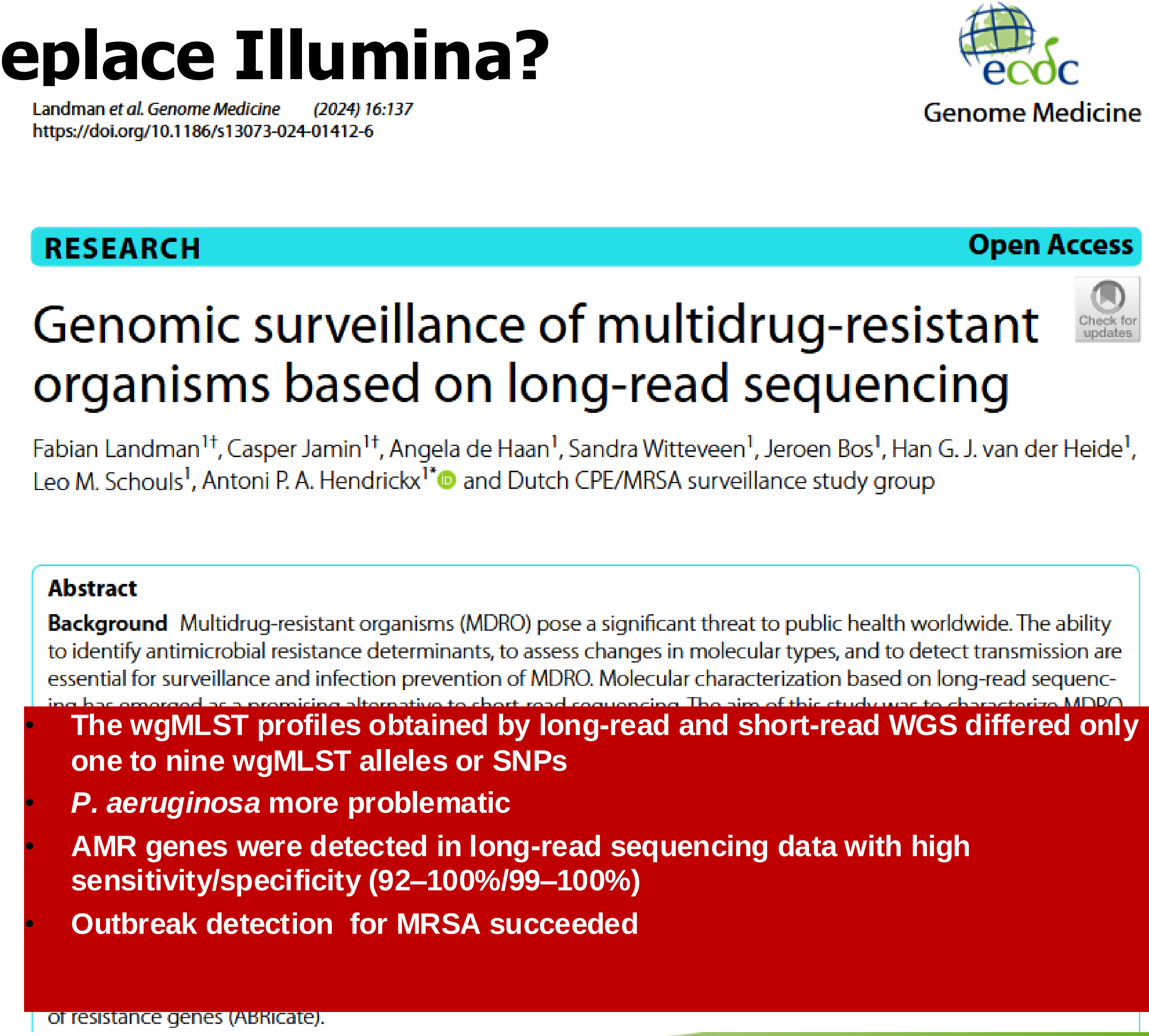
[1]Transversal activities in Applied Genomics, Sciensano, Brussels, Belgium, [2]Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium, [3]National Reference Laboratory for Foodborne Outbreaks (NRL-FBO) and for Coagulase Positive Staphylococci (NRL-CPS), Foodborne Pathogens, Sciensano, Brussels, Belgium, [4]Department of Information Technology, IDlab, IMEC, Ghent University, Ghent, Belgium

https://doi.org/10.3389/fmicb.2024.1330814

Can ONT sequencing replace Illumina?

PH CPE/MRSA study group
- Approx 350 MDRO
  - *Klebsiella pneumoniae*
  - *E. coli*
  - *Enterobacter cloacae compl.*
  - *Citrobacter freundii*
  - *Ps. aeruginosa*
  - *A. baumannii*
  - MRSA

  - MLST, wgMLST, SNP analysis, AMR genes, MRSA typing



Landman *et al. Genome Medicine* (2024) 16:137
https://doi.org/10.1186/s13073-024-01412-6

Genome Medicine

RESEARCH **Open Access**

Genomic surveillance of multidrug-resistant organisms based on long-read sequencing

Fabian Landman[1†], Casper Jamin[1†], Angela de Haan[1], Sandra Witteveen[1], Jeroen Bos[1], Han G. J. van der Heide[1], Leo M. Schouls[1], Antoni P. A. Hendrickx[1*] and Dutch CPE/MRSA surveillance study group

Abstract

Background Multidrug-resistant organisms (MDRO) pose a significant threat to public health worldwide. The ability to identify antimicrobial resistance determinants, to assess changes in molecular types, and to detect transmission are essential for surveillance and infection prevention of MDRO. Molecular characterization based on long-read sequencing has emerged as a promising alternative to short-read sequencing. The aim of this study was to characterize MDRO

of resistance genes (ABRicate).

- **The wgMLST profiles obtained by long-read and short-read WGS differed only one to nine wgMLST alleles or SNPs**
- ***P. aeruginosa* more problematic**
- **AMR genes were detected in long-read sequencing data with high sensitivity/specificity (92–100%/99–100%)**
- **Outbreak detection for MRSA succeeded**

https://doi.org/10.1186/s13073-024-01412-6

Nanopore-only Microbial Isolate Sequencing Solution (NO-MISS)

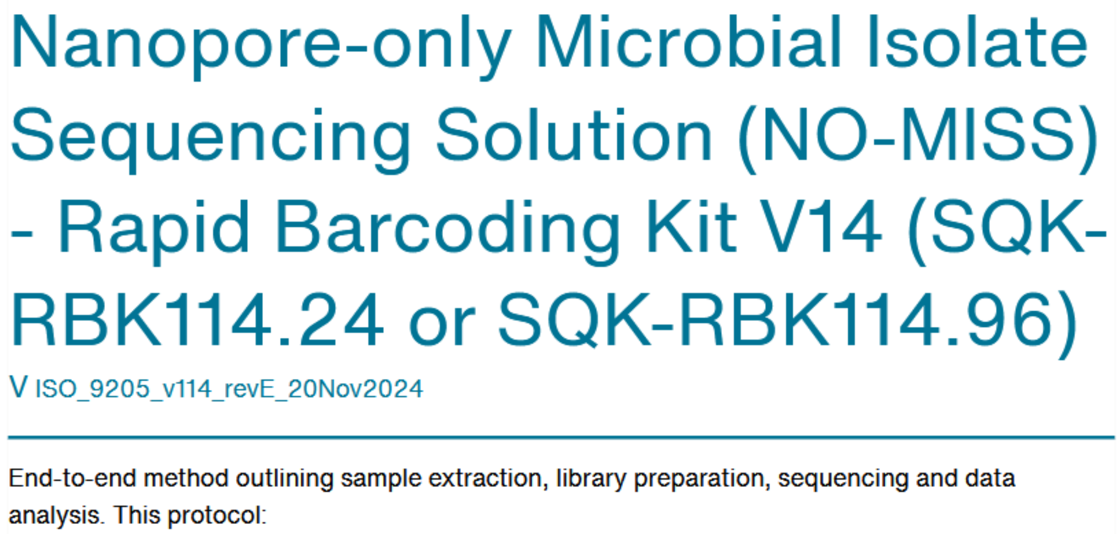
Extraction Methods

Universal bead-beating gDNA extraction for Nanopore-only Microbial Isolate Sequencing Solution (NO-MISS)

Bacteria gDNA extraction for Nanopore-only Microbial Isolate Sequencing Solution (NO-MISS)

Hard to lyse organisms gDNA extraction for Nanopore-only Microbial Isolate Sequencing Solution (NO-MISS)

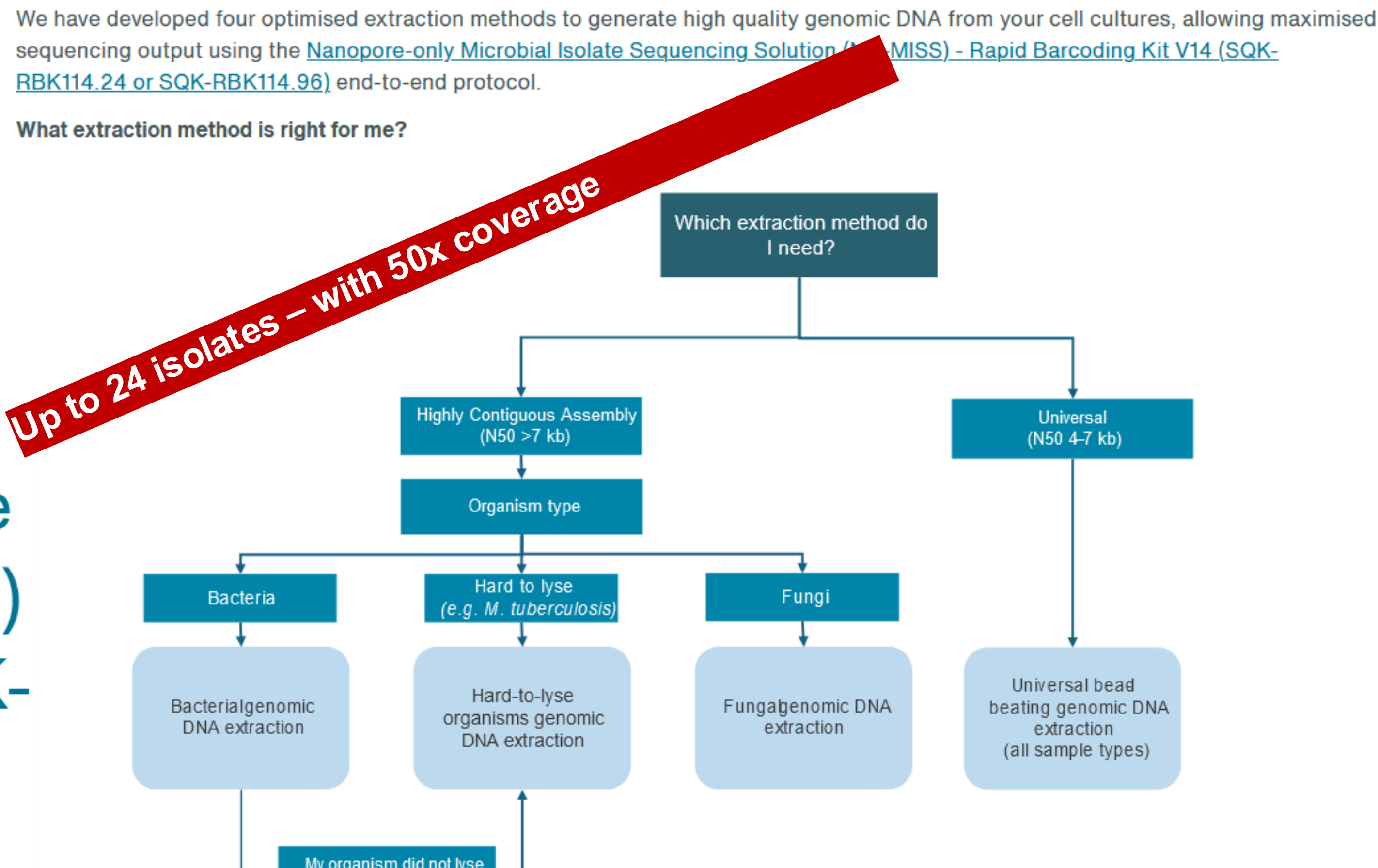
Fungi gDNA extraction for Nanopore-only Microbial Isolate Sequencing Solution (NO-MISS)



Nanopore-only Microbial Isolate Sequencing Solution (NO-MISS) - Rapid Barcoding Kit V14 (SQK-RBK114.24 or SQK-RBK114.96)

V ISO_9205_v114_revE_20Nov2024

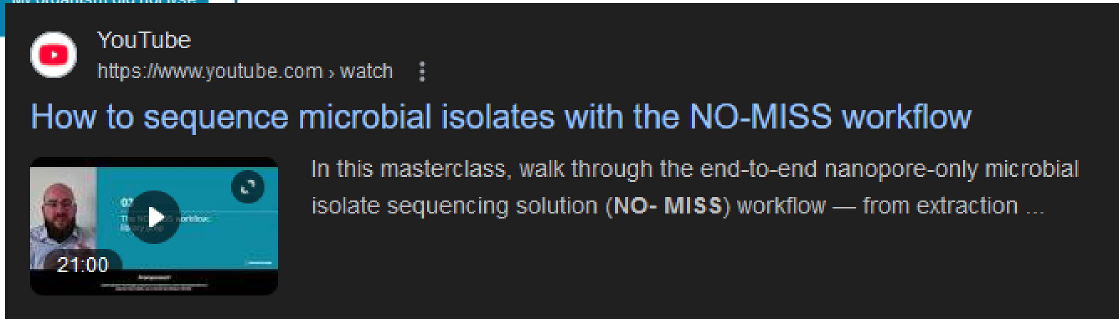
End-to-end method outlining sample extraction, library preparation, sequencing and data analysis. This protocol:



We have developed four optimised extraction methods to generate high quality genomic DNA from your cell cultures, allowing maximised sequencing output using the Nanopore-only Microbial Isolate Sequencing Solution (-MISS) - Rapid Barcoding Kit V14 (SQK-RBK114.24 or SQK-RBK114.96) end-to-end protocol.

What extraction method is right for me?

Up to 24 isolates – with 50x coverage



YouTube
https://www.youtube.com › watch

How to sequence microbial isolates with the NO-MISS workflow

In this masterclass, walk through the end-to-end nanopore-only microbial isolate sequencing solution (**NO- MISS**) workflow — from extraction ...

https://nanoporetech.com/document/no-miss-isolate-sequencing-rapid-barcoding-v14

Press release

New study shows MHRA collaboration with hospital DNA sequencing service cuts time to diagnose infections

In a UK-first, the Medicines and Healthcare products Regulatory Agency (MHRA) and Barts Health NHS Trust have developed a DNA sequencing approach that can be implemented onsite in hospitals so they can diagnose bacterial infections faster and more accurately.

https://www.gov.uk/government/news/new-study-shows-mhra-collaboration-with-hospital-dna-sequencing-service-cuts-time-to-diagnose-infections

This technology is now being piloted to investigate and prevent hospital outbreaks caused by antibiotic-resistant ‘superbugs’ — a growing global threat.

Standardization of 16S rRNA gene sequencing using nanopore long read sequencing technology for clinical diagnosis of culture negative infections

Ian Butler[1*] Olivia Turner[1] Kulsoom Mohammed[1] Mazeda Akhtar[1]
Daniel Evans[2†] Jonathan Lambourne[1†] Kathryn Harris[1†]
Denise M. O'Sullivan[2,3†] Chrysi Sergaki[4†]

[1] Department of Microbiology, National Health Services (NHS) East and South East London Pathology Partnership, Bart's Health NHS Trust, London, United Kingdom
[2] Molecular and Cell Biology, National Measurement Laboratory (NML), Laboratory of the Government Chemist (LGC), London, United Kingdom
[3] School of Biosciences & Medicine, University of Surrey, Guildford, United Kingdom
[4] Medicines and Healthcare Products Regulatory Agency (MHRA), London, United Kingdom

doi.org/10.3389/fcimb.2025.1517208

Beyond DNA sequencing

applications of nanopores in

- molecular sensing and sequencing, chemical catalysis and biophysical characterization.
- single-protein analysis and sequencing, single-molecule covalent chemistry,
- clinical sensing applications for single-molecule liquid biopsy,
- synthetic biomimetic nanopores as experimental models for natural systems.

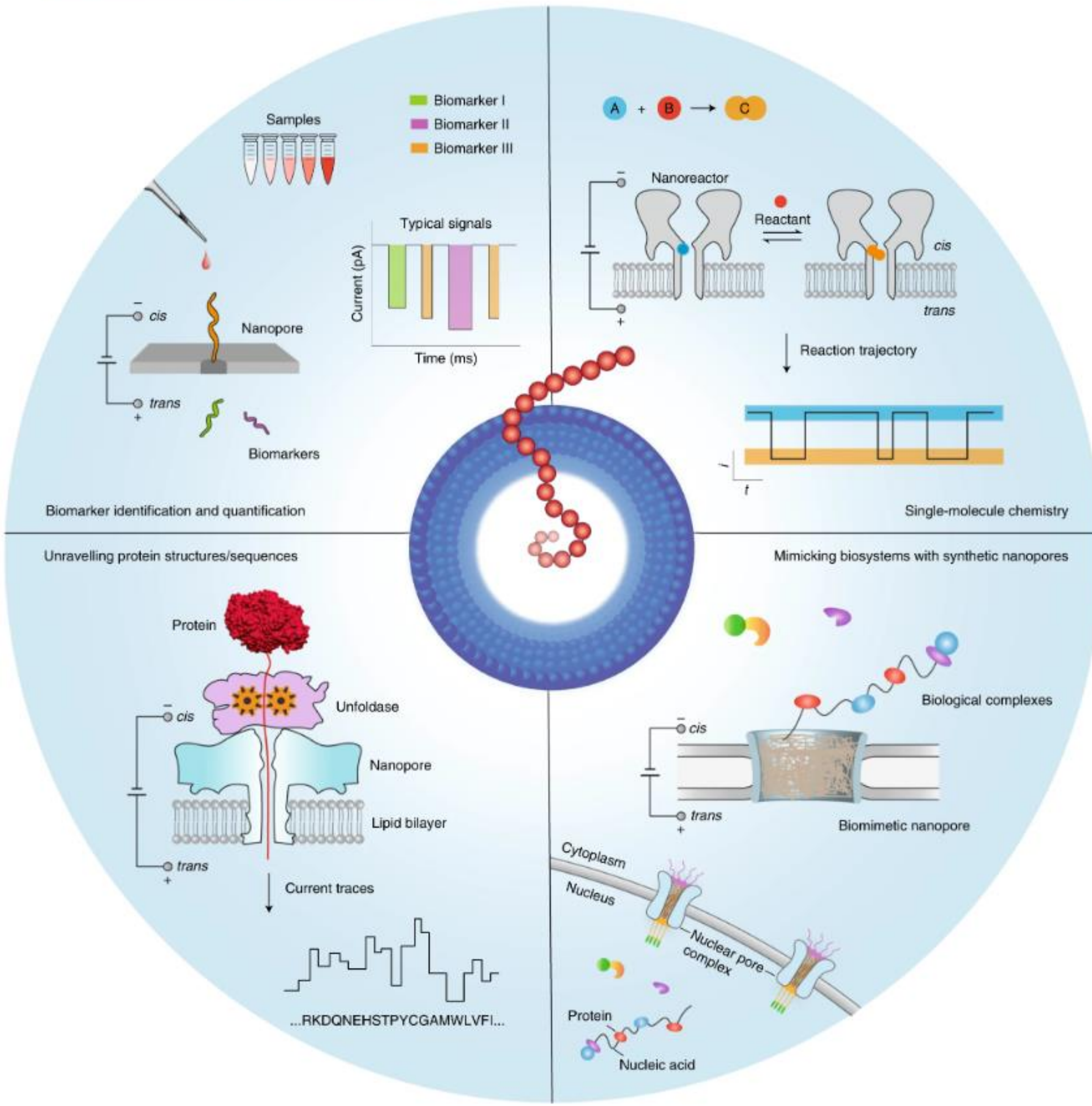
Nanopore-based technologies be

Yi-Lun Ying, Zheng-Li Hu, Shengli Zhang, Yujia Qing, Alessio Fra

Hagan Bayley ✉, Cees Dekker ✉ & Yi-Tao Long ✉

Nature Nanotechnology **17**, 1136–1146 (2022) | Cite this article

From: Nanopore-based technologies beyond DNA sequencing



Links for resources and literature:

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Nanopore protocols, white papers and overview of publications etc:

https://nanoporetech.com/resource-centre

Webinars

- https://nanoporetech.com/resource-centre/webinar-onramp-rapid-multiplexed-validation-plasmids-using-nanopore-sequencing
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- https://doi.org/10.1016/j.ygeno.2019.04.006
- https://doi.org/10.1101/2023.10.25.563907
- doi: 10.1093/gigascience/gix132
- https://doi.org/10.1186/s12859-023-05226-y
- doi:10.1099/mgen.0.001024
- doi: 10.1099/mgen.0.000631

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