

Ordering My Transcripts is Surprisingly Expensive

(Not my real title)

3BP3 TA Talk

October 8, 2024

BSc – Microbiology University of Guelph



North Pacific Ocean

Bering Sea

Gr

Labrador Sea

Northwestern
Passages

Hudson Bay

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North Pacific Ocean

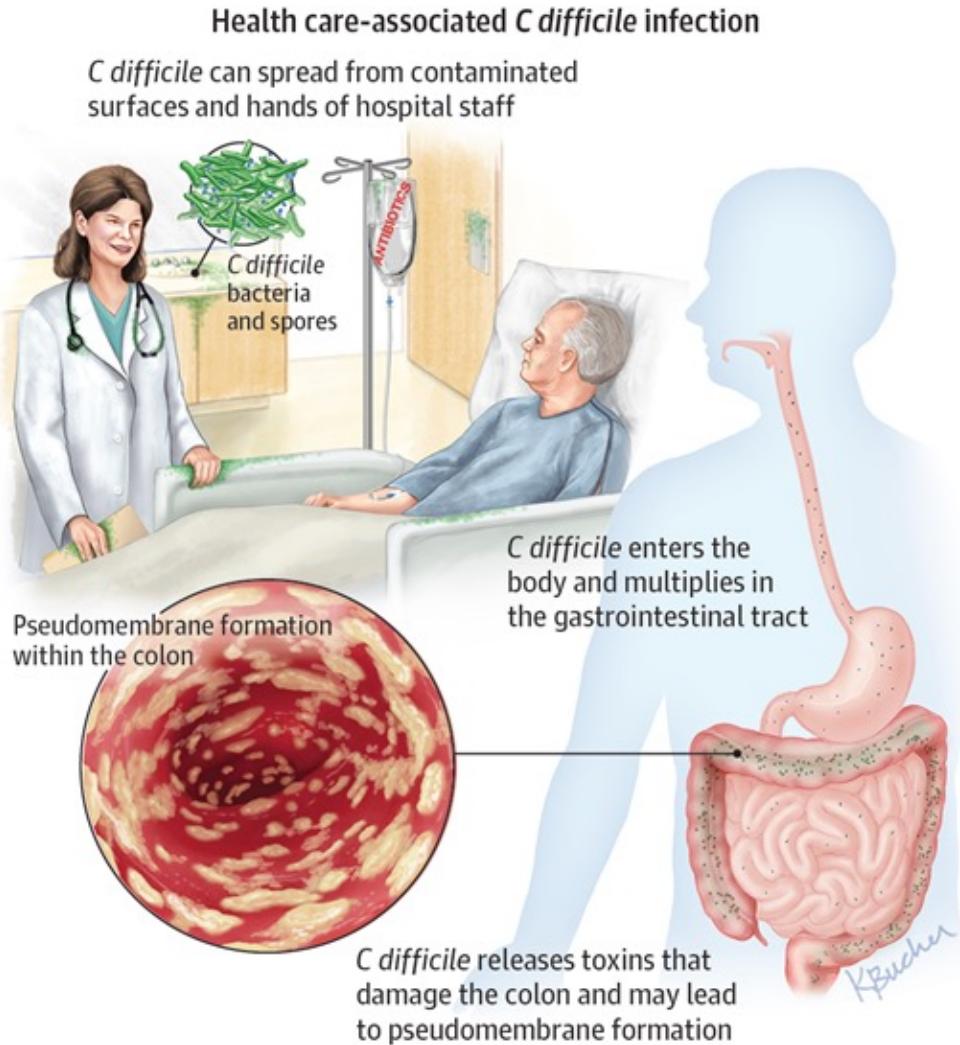
<p

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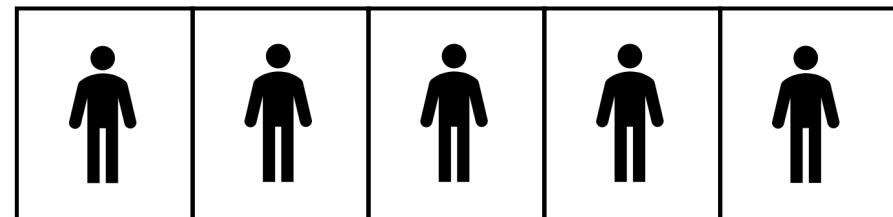
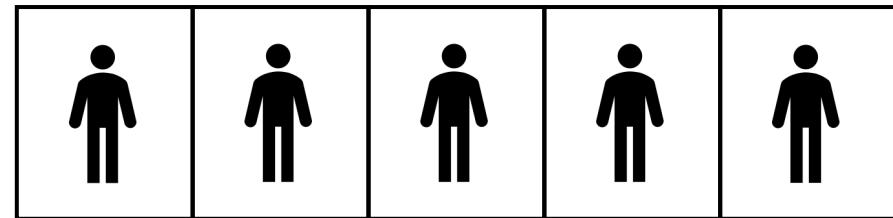
MSc – Microbiology and Immunology University of Saskatchewan (VIDO)



Culture-free Outbreak Detection and Surveillance



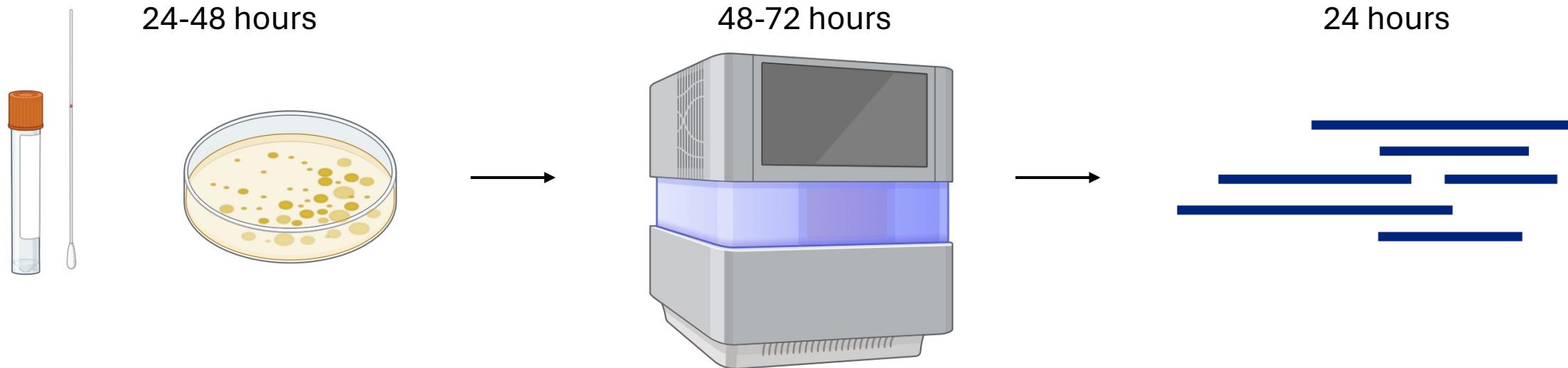
HOSPITAL WARD



- Rapid diagnosis is simple
- Outbreak detection requires significantly more work

How are *C. diff* Outbreaks identified?

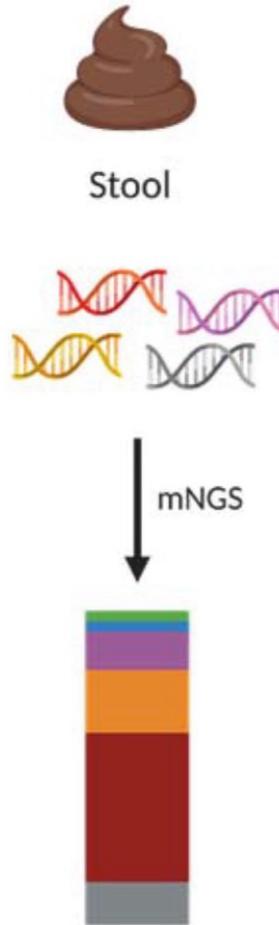
A minimum of 4 days to culture and sequence all potential outbreak cases



Some may not grow well in culture

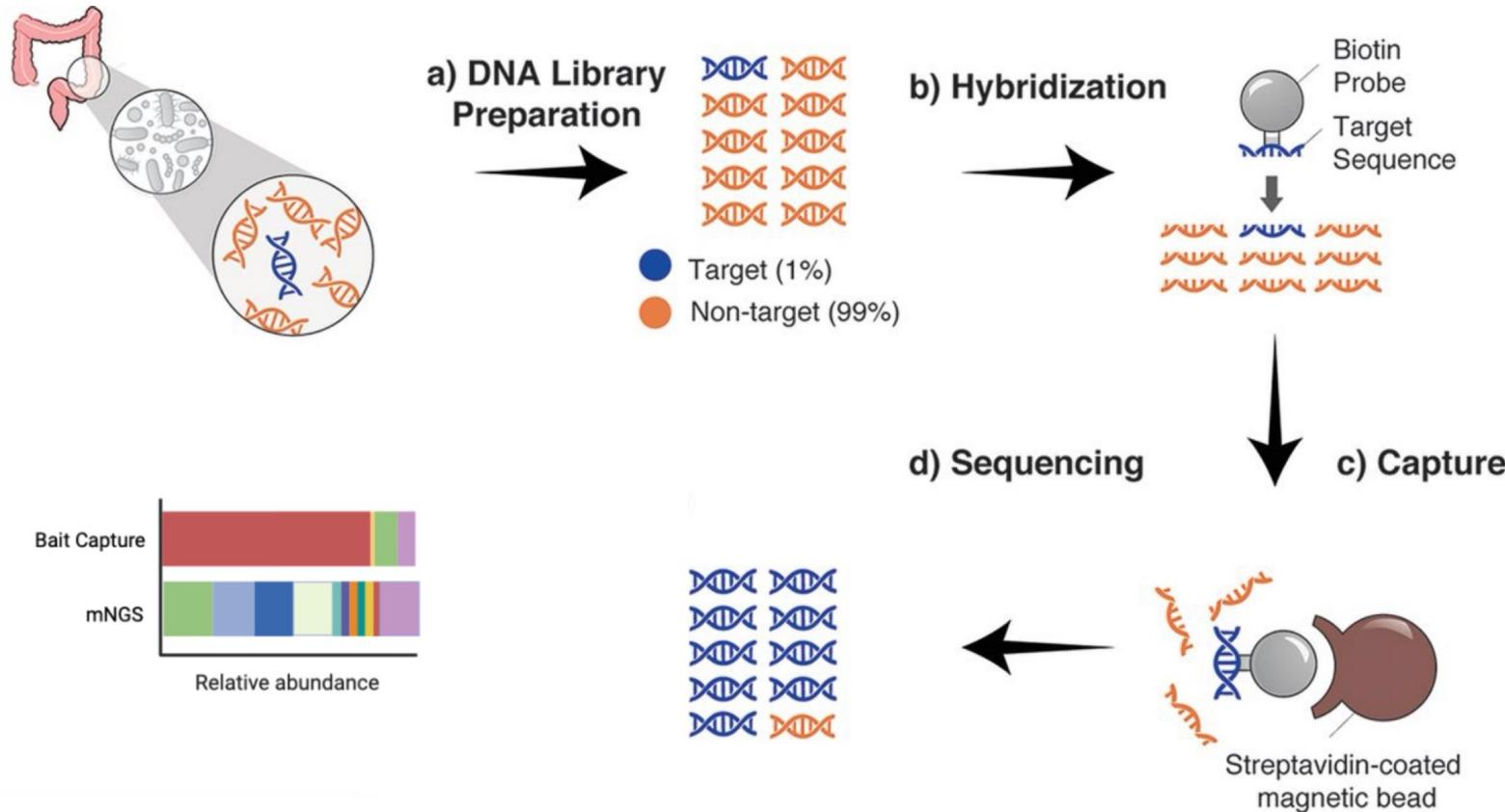
May miss multi-strain infections

Shotgun metagenomics as an alternative

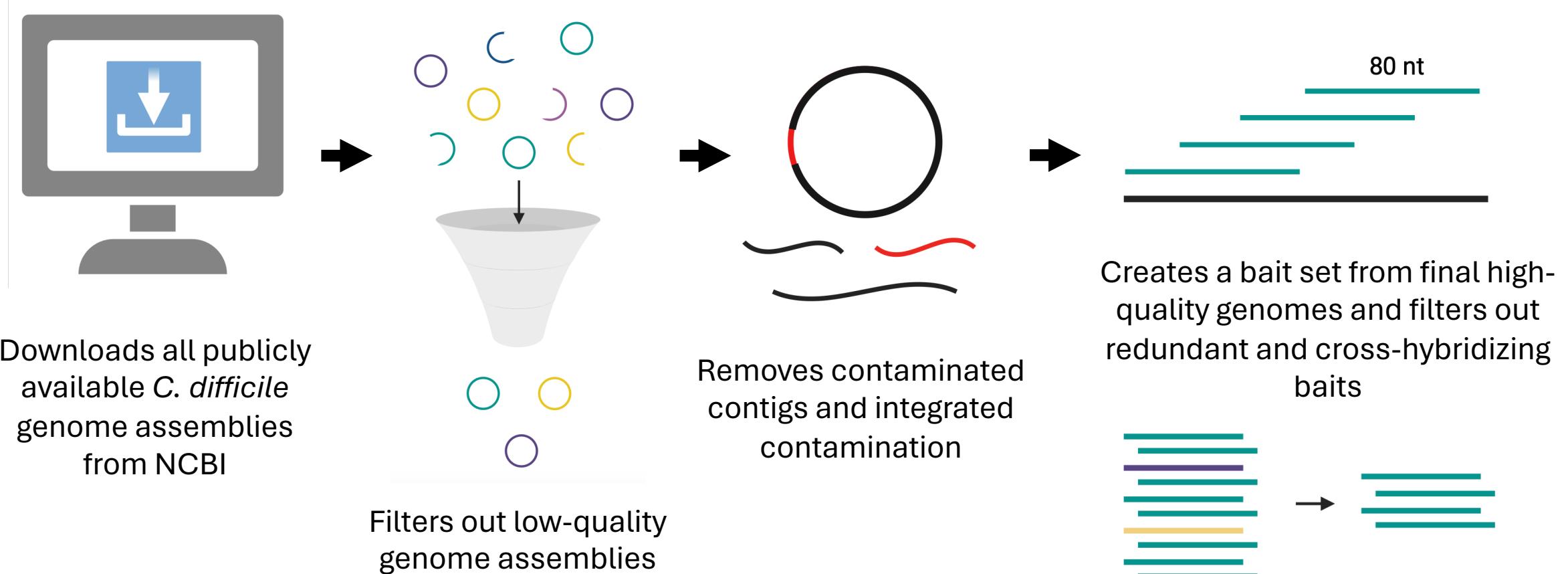


- Some *C. difficile* patients don't shed large amounts of *C. diff* DNA in their stool
- Pathogen DNA may be vastly outnumbered by DNA fragments from members of the microbiome
- Requires deeper sequencing to recover the whole pathogen genome
- **Time and \$\$\$**

Bait Capture “enriches” for target sequences in a complex sample

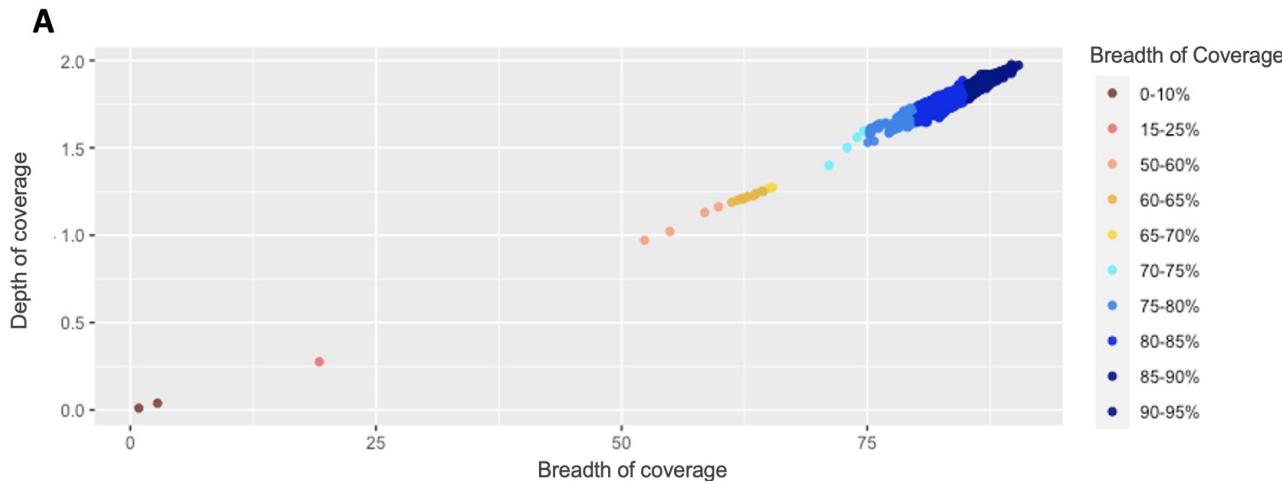


Capturing *C. difficile* Outbreaks With Baits



Limitations of Bait Capture

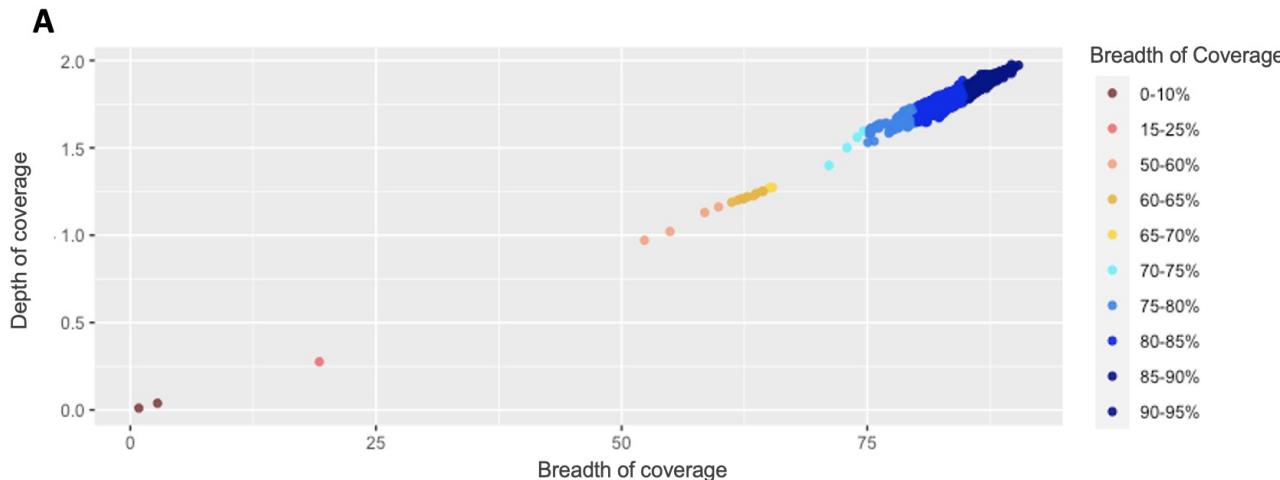
Breadth and Depth of Coverage of *C. difficile* Bait Set



- *In silico* prediction shows high coverage across 17,000 *C. difficile* genomes
- Even with cost-cutting measures, *C. diff* bait set still costs > \$20/sample

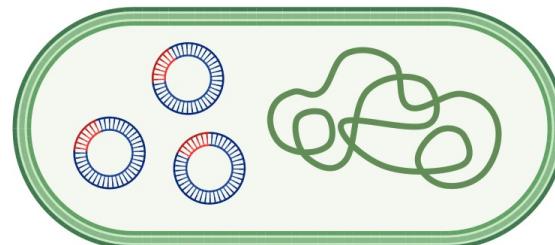
Limitations of Bait Capture

Breadth and Depth of Coverage of *C. difficile* Bait Set



- *In silico* prediction shows high coverage across 17,000 *C. difficile* genomes
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- Difficult to design bait sets to capture closely related species
- Clinically relevant plasmids?



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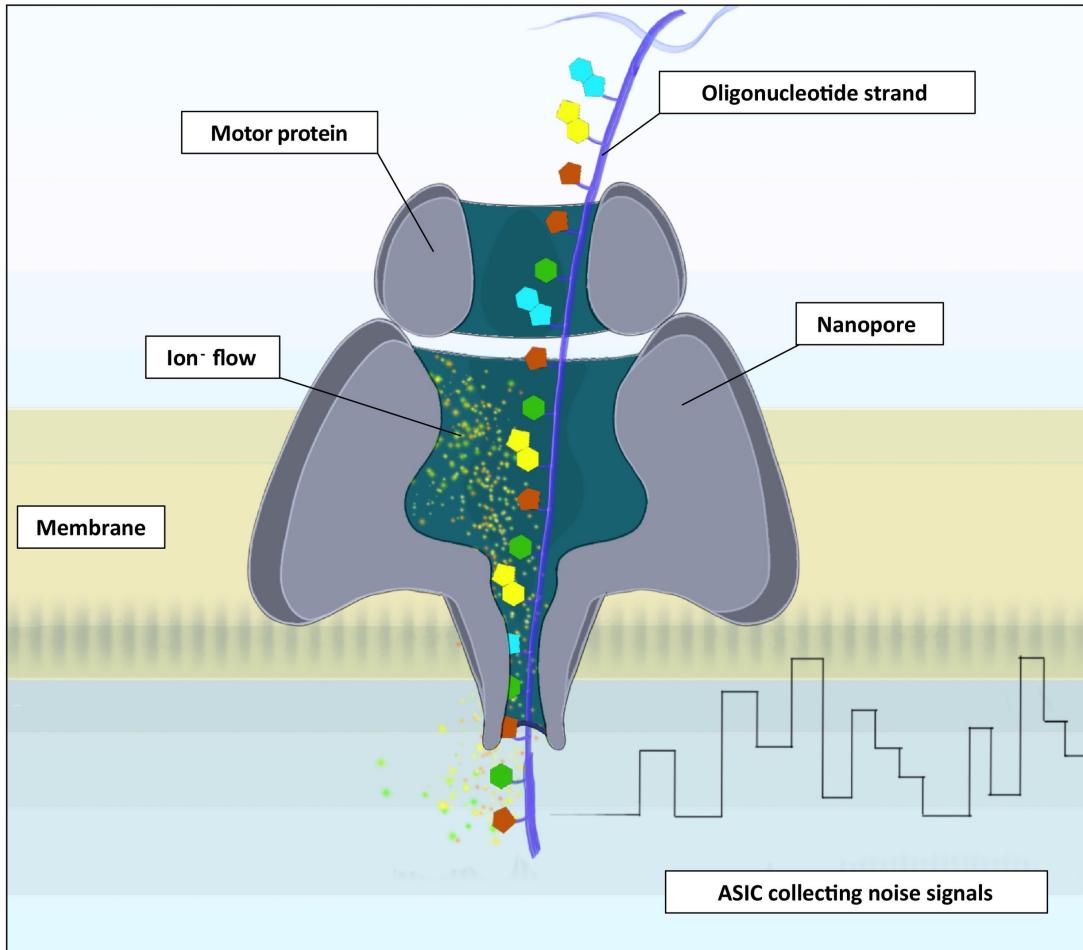
Plasmid Acquisition Alters Vancomycin Susceptibility in *Clostridioides difficile*

Meng Pu¹ • Janice M. Cho¹ • Scott A. Cunningham² • ... • Gary Dunny⁶ • Robin Patel^{2,7} • Purna C. Kashyap⁸

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Affiliations & Notes ▾ Article Info ▾

Current and Future Directions: Long Read Sequencing



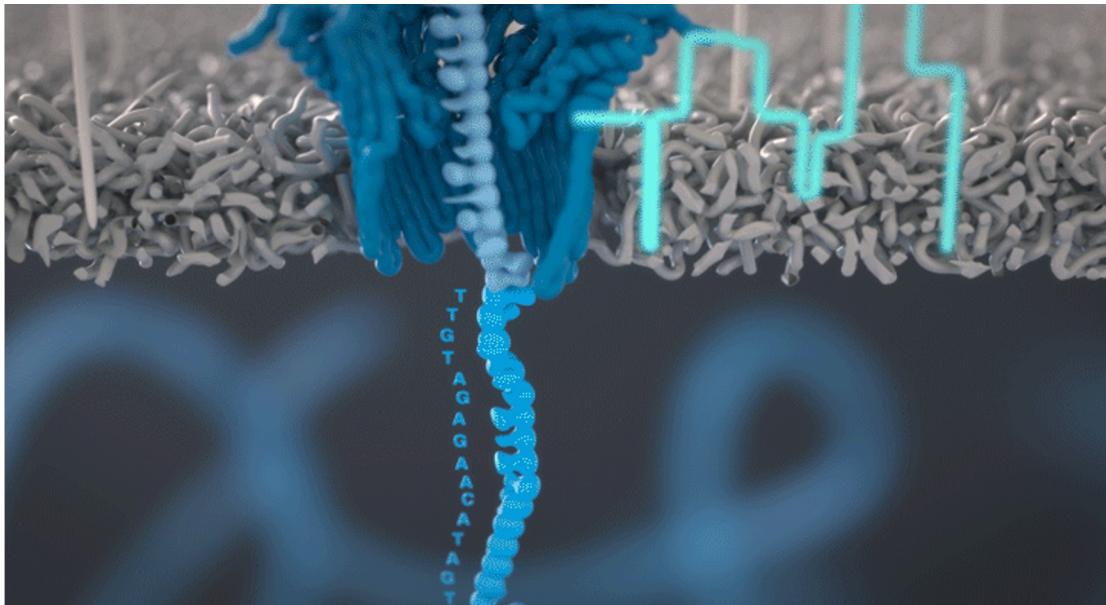
- DNA fragments move through a protein pore embedded in the flow cell membrane
- As each base goes through the pore it disrupts the electrochemical current in a unique way
- Those signal disruptions are converted to DNA bases by Nanopore software
- Significantly longer reads compared to Illumina sequencing

Nanopore Adaptive Sampling

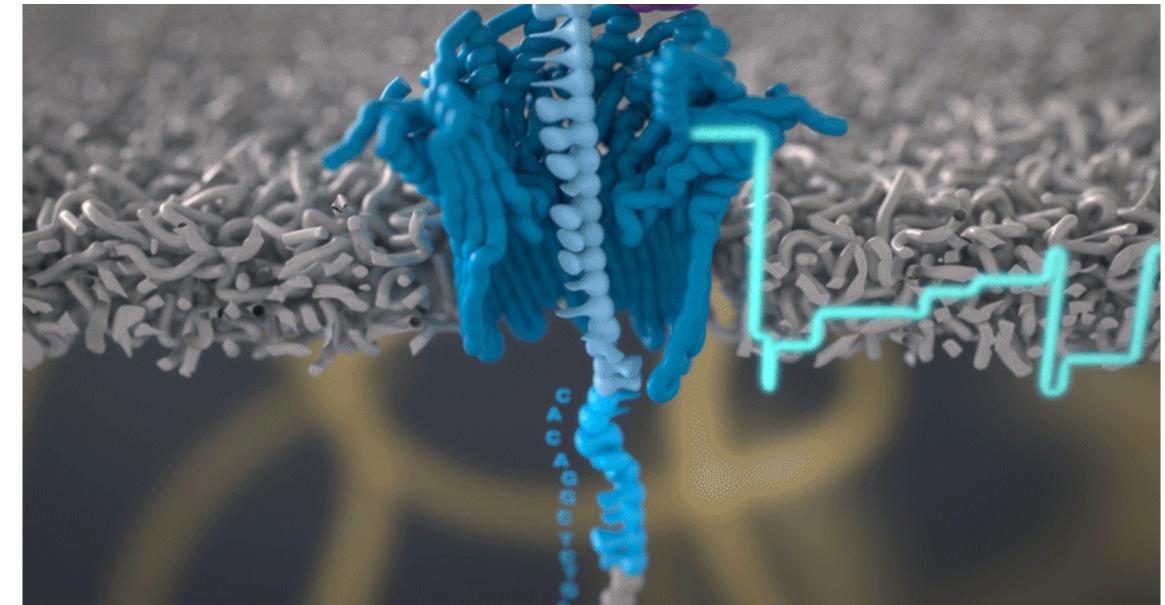
Nanopore Adaptive Sampling (NAS) uses software-based enrichment to selectively sequence target DNA fragments

User-provided FASTA file of sequences to select

Match - Keep



No Match - Reject

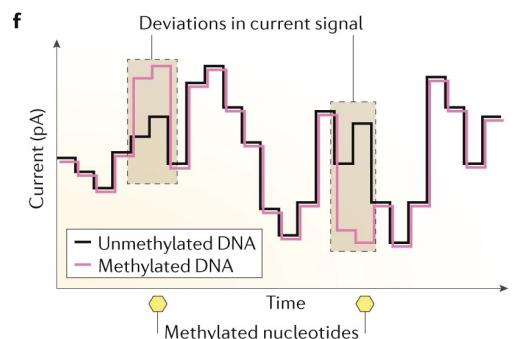
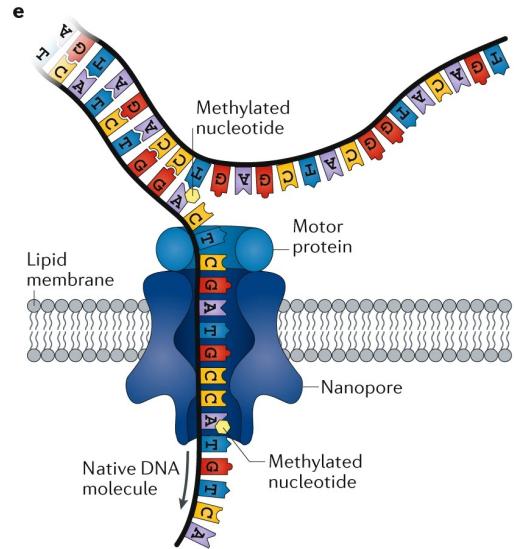
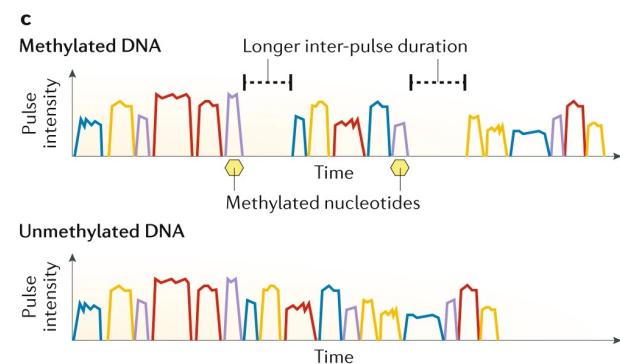
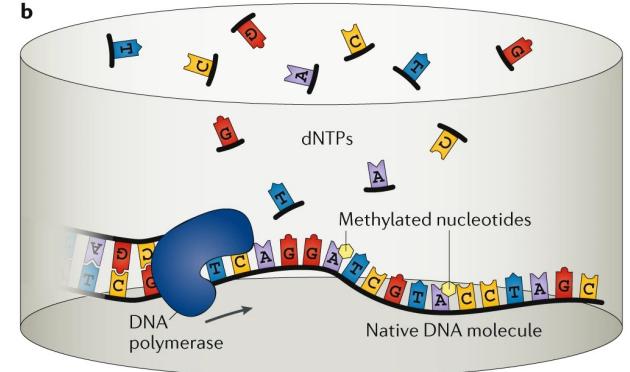
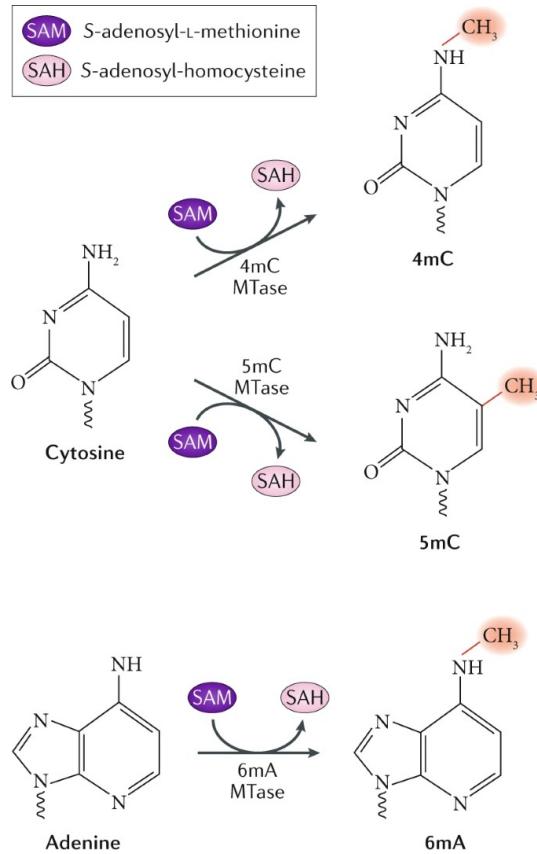


Significantly cheaper than designing multi-pathogen bait sets

Much simpler laboratory protocol – simpler to train clinical staff

Methylation Detection

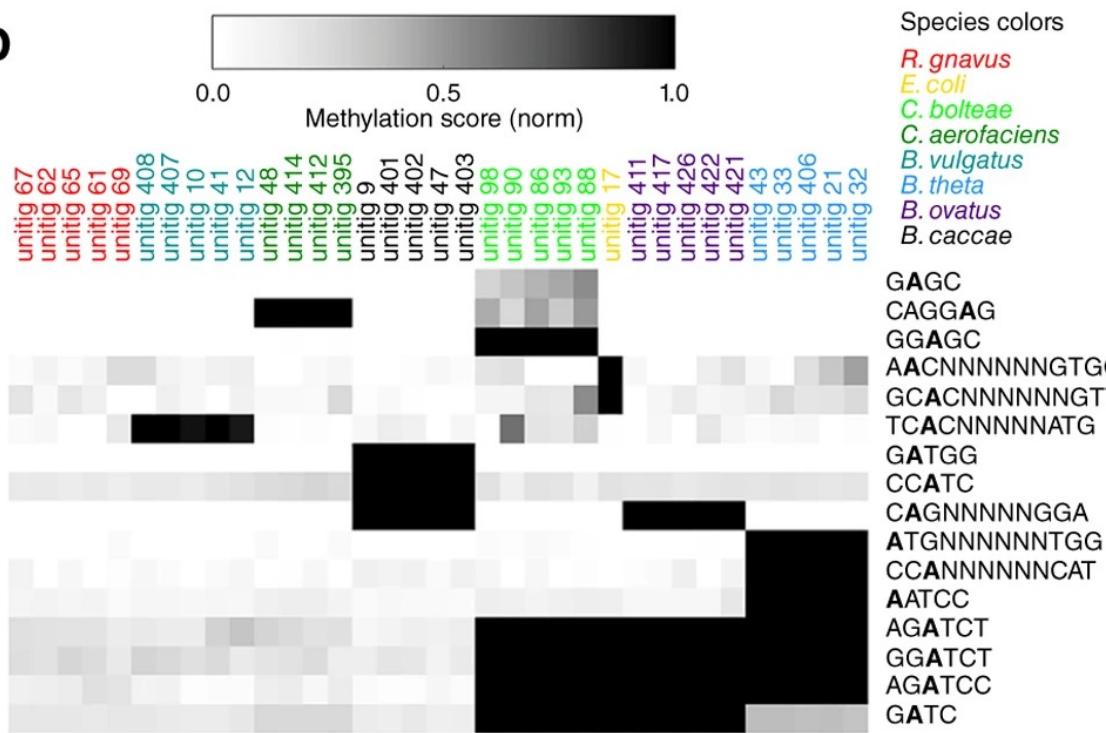
All DNA in a cell is methylated by the same enzyme, giving it a characteristic pattern



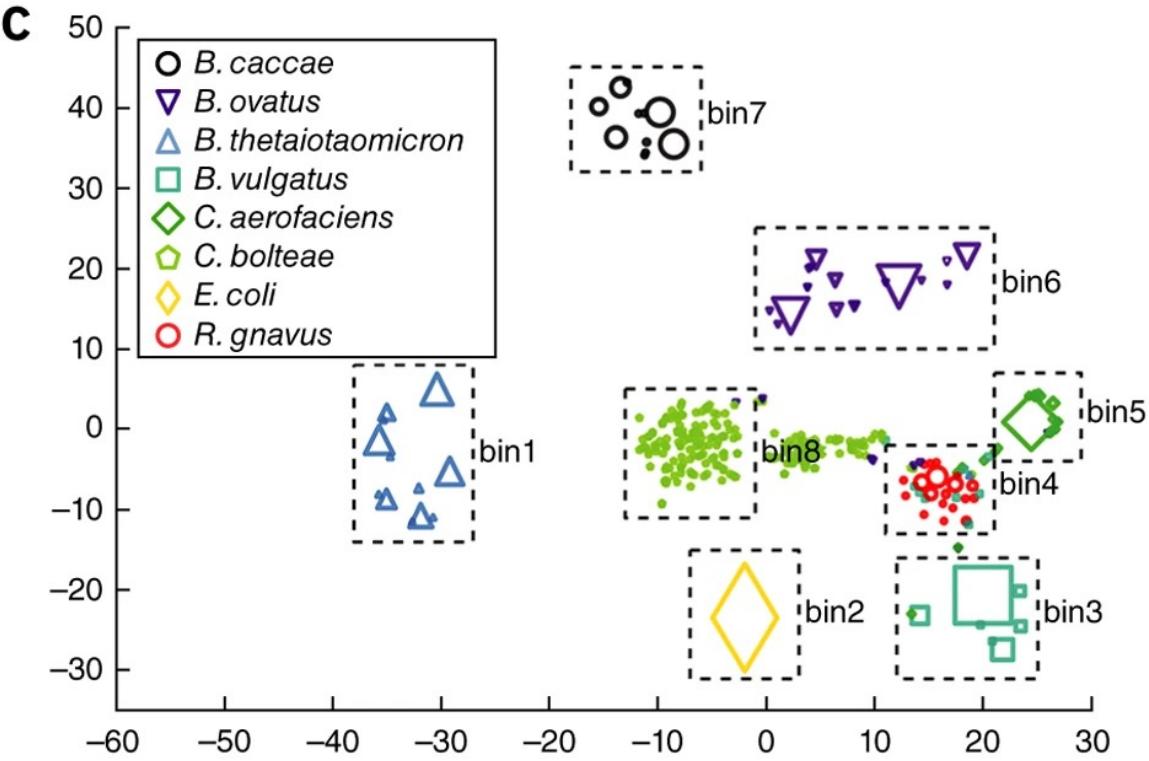
Methylation Detection

Sequenced reads (including plasmids) can be grouped together based on methylation patterns

b



c



Methylation and Virulence

- Methylomics - emerging research studying methylation signatures and virulence
- Culture-based sequencing alters methylation patterns compared to active infection

[Nat Microbiol. 2020 Jan; 5\(1\): 166–180.](#)

Published online 2019 Nov 25. doi: [10.1038/s41564-019-0613-4](https://doi.org/10.1038/s41564-019-0613-4)

PMID: [31768029](#)

Epigenomic characterization of *Clostridioides difficile* finds a conserved DNA methyltransferase that mediates sporulation and pathogenesis

Pedro H. Oliveira,¹ John W. Ribis,² Elizabeth M. Garrett,³ Dominika Trzilova,³ Alex Kim,¹ Ognjen Sekulovic,²

ORIGINAL RESEARCH article

Front. Fungal Biol., 14 January 2021

Sec. Fungi-Plant Interactions

Volume 1 - 2020 | <https://doi.org/10.3389/ffunb.2020.614633>

DNA Methylation Is Responsive to the Environment and Regulates the Expression of Biosynthetic Gene Clusters, Metabolite Production, and Virulence in *Fusarium graminearum*

JOURNAL ARTICLE

Phase variation of DNA methyltransferases and the regulation of virulence and immune evasion in the pathogenic *Neisseria*

Kate L. Seib , Freda E.-C. Jen, Adeana L. Scott, Aimee Tan, Michael P. Jennings 

Pathogens and Disease, Volume 75, Issue 6, August 2017, ftx080,

<https://doi.org/10.1093/femspd/ftx080>

Published: 14 July 2017 Article history ▾

[Microbiology and Infectious Disease](#)

DNA Methylome Regulates Virulence and Metabolism in *Pseudomonas syringae*

Jiadai Huang, Fang Chen, Beifang Lu, Yue Sun, Youyue Li, Canfeng Hua, Xin Deng 

Department of Biomedical Sciences, City University of Hong Kong, 83 Tat Chee Rd, Kowloon Tong, Hong Kong, China • Shenzhen Research Institute, City University of Hong Kong, Shenzhen, Guangdong, China • Tung Research Centre, City University of Hong Kong, Hong Kong SAR, China ... [show 1 more](#)

<https://doi.org/10.7554/eLife.96290.1> 