INSTITUT DE BIOLOGIE INTÉGRATIVE ET DES SYSTÈMES
IBIS

Oxford NANOPORE Technologies

UNIVERSITÉ LAVAL
Faculté de médecine

Harnessing the power of Oxford Nanopore long-read DNA and RNA sequencing for innovative strategies in analysis of non-model organisms in ecogenomics and disease

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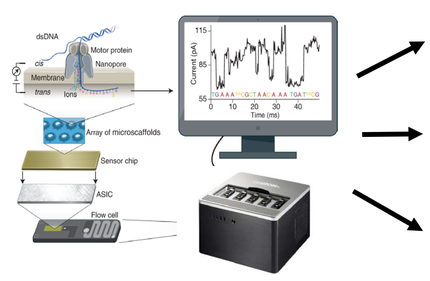
BACKGROUND & STRATEGY

- Oxford Nanopore long-read DNA-RNA (lrDNA and lrRNA) sequencing is now available to all small research laboratories in terms of cost, accessibility, and genome analysis. Coupled to a repertoire of software genome assemblers and filter-processing tools, Oxford Nanopore long read DNA-RNA sequencing can deliver chromosome level assemblies of non-model organisms.

- This Nanopore disruptive OMICS technology has been used in our laboratory for a series of six projects: 1) Surveillance of 6 enteropathogens and COVID-19 in wastewater; 2) Carbapenem resistance; 3) Lyme disease and *Borrelia* genomic-plasmid diversity; 4) Assembly of the 570 Mb 30 chromosome spruce budworm genome; 5) Rapid on-site detection of cyanobacterial blooms; 6) The quest for novel antimicrobials using iChip technology to culture and isolate novel microorganisms from wild and untapped geographic regions.

Oxford Nanopore long-read DNA-RNA sequencing



Whole genome sequencing (WGS)

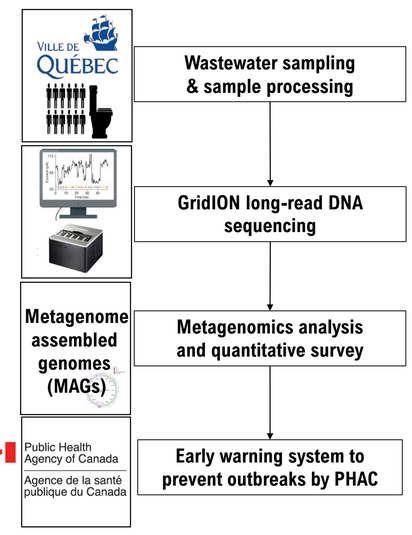
Metagenome assembled genomes (MAGs)

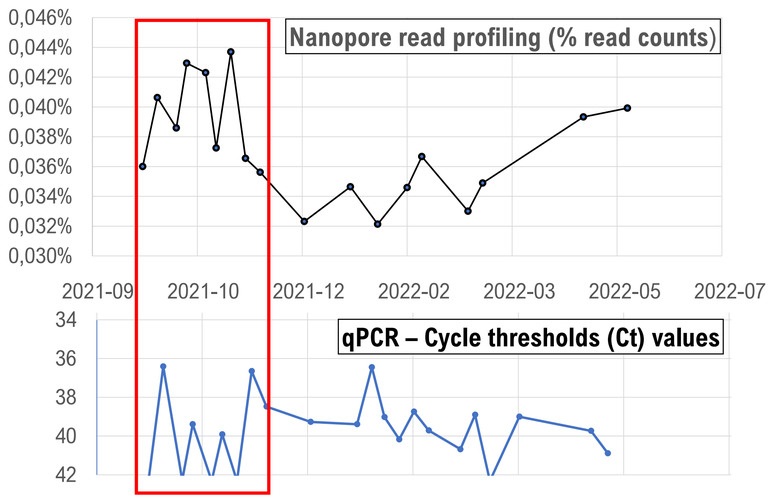
Direct RNA sequencing

ENTEROPATHOGEN AND ANTIMICROBIAL RESISTANCE SURVEILLANCE IN WASTEWATER AND CLINICAL SAMPLES

1) SENTINEL: Stopping Enteric Illnesses Early

- **Foodborne pathogen surveillance in wastewater (*Salmonella, E. coli O157-H7, Campylobacter, Vibrio, Listeria*)**
- **Targeted metagenomics, social media monitoring via machine learning**
- **Transfer to Public Health Agency of Canada (PHAC) for early warning system to prevent outbreaks**

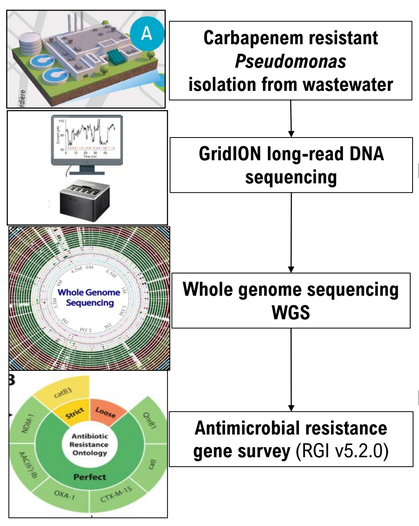


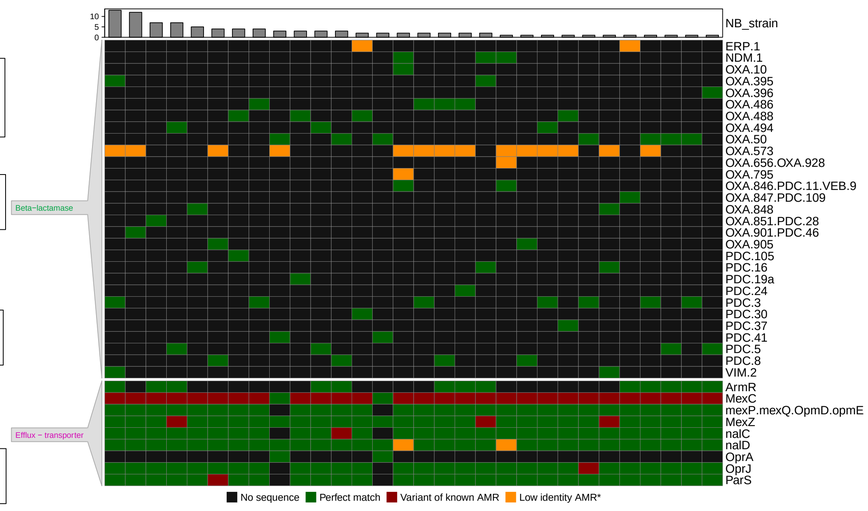


Advanced detection of a nationwide *Salmonella* outbreak prior to governmental agency recall October 27th 2021.

2) SAMPAN: A Smart Surveillance Strategy for Carbapenem resistant *P. aeruginosa*

- **Carbapenem resistant Pseudomonas surveillance in wastewater (Netherlands, Italy, Indonesia, Quebec/Canada)**



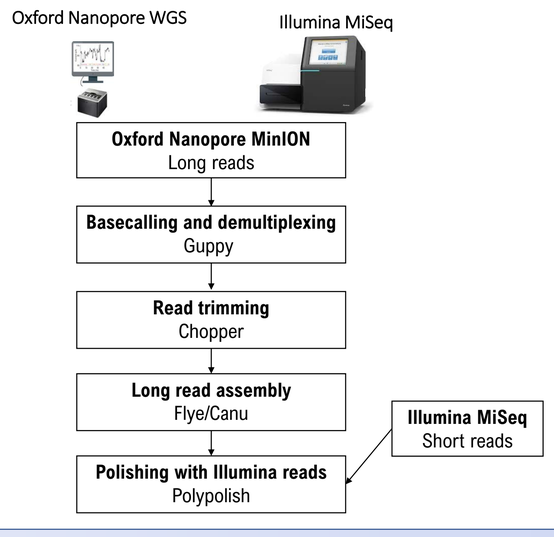


Heatmap identification of carbapenem-resistant *P. aeruginosa* from clinical and wastewater isolates

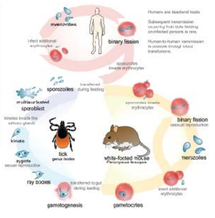
ECOGENOMICS OF ZOONOSES AND INSECTS

3) Systems Biology Ticks, *Borrelia* & Lyme Disease

Hybrid assembly workflow used for *Borrelia* genomes



Adult and nymph black tick
Ixodes scapularis 2 100 Mb genome



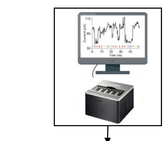
Borrelia burgdorferi
spirochaete, 0.9 Mb genome & 5-20 plasmids

Hybrid assembly statistics of five Canadian strains of *Borrelia burgdorferi*.

Strain	Contigs	Genome size	Longest contig
Bb16-134	15	1.53 Mb	943.8 Kb
Bb16-150	22	1.71 Mb	909.4 Kb
Bb16-15-2	17	1.53 Mb	942.7 Kb
Bb16-183	27	1.79 Mb	939.9 Kb
Bb16-23-2	13	1.36 Mb	909.3 Kb

4) Spruce budworm, *Choristoneura fumiferana*

- **Most devastating insect pest of the boreal forest in eastern Canada**



Pooled ONT reads
15.5 Gb (out of 18 Gb total)
25 X genome coverage

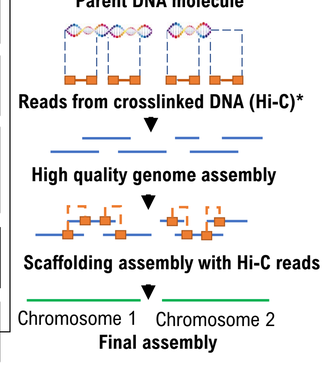
Assembly
Canu

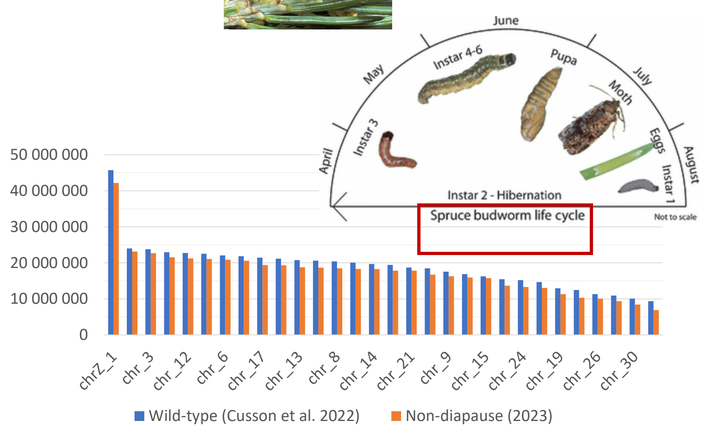
Polish with MiSeq short reads
Pilon

Scaffolding
LONGSTITCH

Final polishing
Pilon

Guided scalfolding with Hi-C data

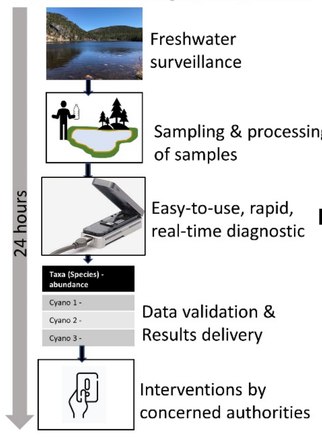


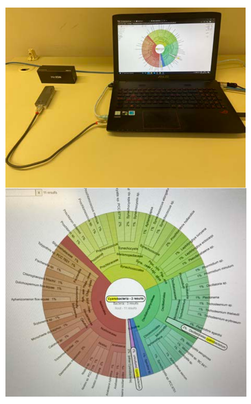


Comparative chart of chromosome lengths between male *Choristoneura fumiferana* wild-type genome (blue) and non-diapause (orange). Chromosomes are ordered by size, not by numeral order.

ENVIRONMENTAL MONITORING AND BIOPROSPECTION FOR NOVEL ANTIMICROBIALS

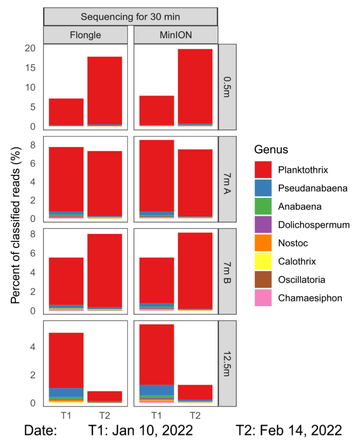
5) RosHAB: Rapid On-Site detection of cyanobacterial Harmful Algal Blooms





Hardware:
- 16 GB RAM
- 8 processors
- 1 TB hard drive
- Windows 10
- MinION (USB)

Software:
- MinKNOW (sequencing)
- Kraken2 (identification)
  - Cyano DB (2GB)



Relative abundance genera at different depths with two flowcells Flongle vs MinION after 30 minutes.

6) Isolation Chips (iChips)

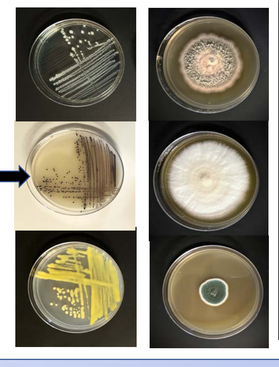
Camp Lake (BYL37):
Bylot Island, Nunavut, Canada
N 73.152970 W 79.977178

Sampling using iChips

Collection of iCHIP isolates

Whole genome sequencing:
Oxford Nanopore
Illumina MiSeq

Direct RNA Sequencing



Complete genome sequencing and assembly of iChip bacteria and fungi isolates.

iCHIP isolate	Scaffolds	Genome Size (Mb)	Classification
4-I-48	4	5,66	***Arthrobacter***
4-R-4-2	1	4,76	***Chryseobacterium***
7-A-59	1	5,67	***Flavobacterium***
4-A-20	2	6,31	***Janthinobacterium***
7-A-46	1	6,12	***Pedobacter***
4-I-8-2	1	6,94	***Pseudomonas***
4-A-56	1	6,46	***Pseudomonas antarctica***
4-I-8-1	2	4,93	***Specibacter***
Fungi A	22	32,0	**Hypocreales**
Fungi B	53	33,6	**Pleosporales**
Fungi C	24	32,1	**Hypocreales**
Fungi D	30	34,1	**Pleosporales**
Fungi E	34	31,0	**Cladosporiales**
Fungi F	28	30,9	**Cladosporiales**

CONCLUSIONS & PERSPECTIVES

- In a human and animal one health strategy, these OMICS lrDNA and lrRNA genome analysis on-site and in real-time will allow health authorities to establish early warning of emerging pathogens, antimicrobial resistance, and optimal therapeutics.

- Oxford Nanopore lrDNA and lrRNA sequencing for wastewater monitoring and surveillance using WGS and MAGs will orient earlier interventions and prevent outbreaks.

- Ecogenomics strategies using Nanopore lrDNA and lrRNA data coupled to machine learning ecology will pave the way to study the evolution of globally dispersed pathogens (zoonotic risk assessment) and disruptive strategies for novel therapeutics.

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