RPC Journey to ISO/IEC 17025 Accreditation of MSX/SSO/Dermo qPCR Assays and deeper understanding of Oyster disease

Funded projects, pearls of progress.

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Supervisor of Fish Health Services
November 10, 2025

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NBFF Funded Project Overview

- *“ Development and validation of a multiplex quantitative polymerase chain reaction (qPCR) for the detection and quantification of three parasites. Haplosporidium nelson (MSX), Haplosporidium costale (SSO) and Perkinsus marinus (Dermo).”*

NBFF Funded Project Description

Project proposal points:

- Addition of a real-time PCR platform
- Addition of a digital real-time PCR platform
- Development of a multiplex real-time PCR for the detection of MSX, SSO and Dermo
- Improved capacity to deliver high throughput testing.

NBFF Project Objectives

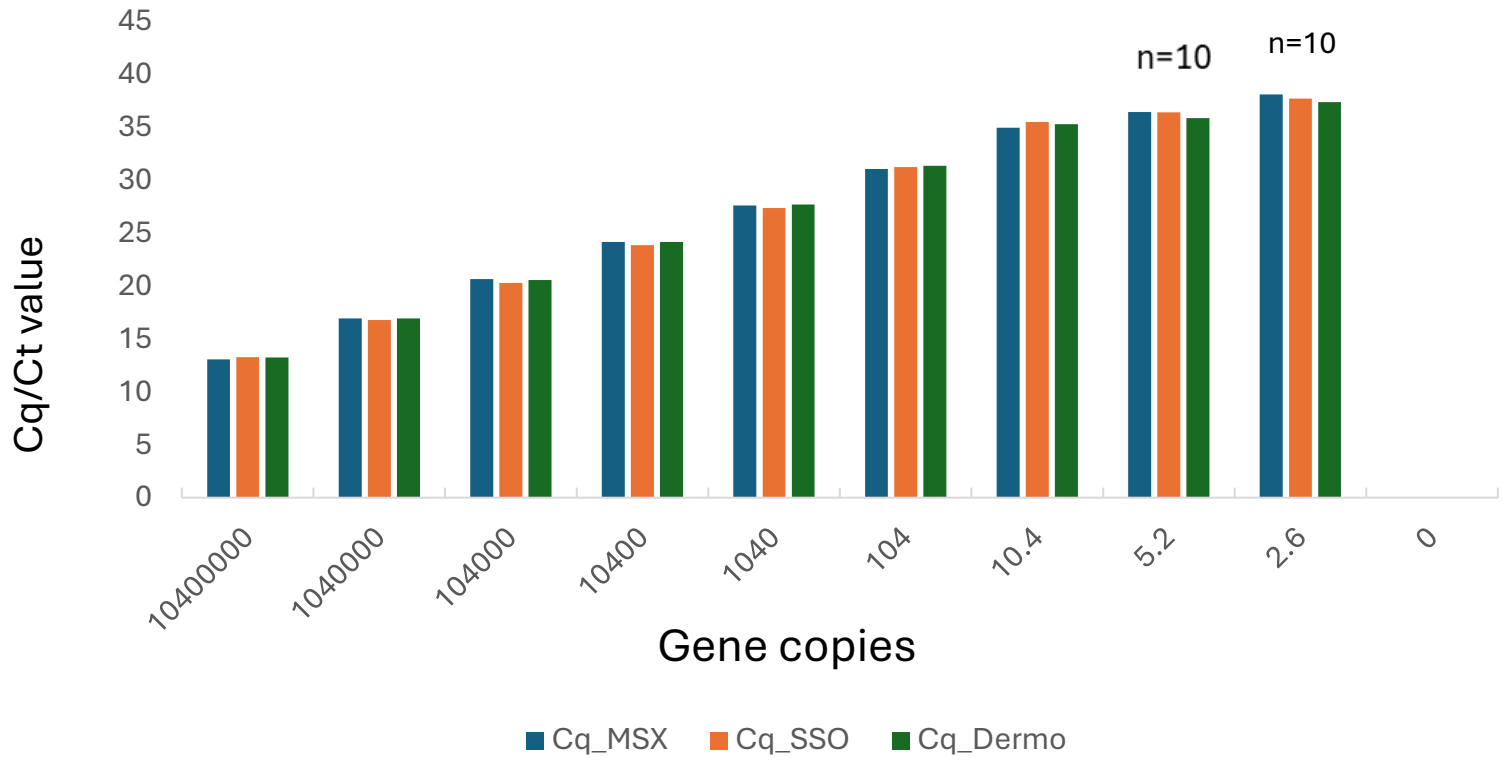
- Validation of Multiplex qPCR for MSX, SSO and Dermo
- Comparison of QuantStudio real-time PCR and Absolute Q Digital PCR (dPCR)
- Prepare method validation package to add to the scope of accreditation for ISO 17025
- Rapid service to industry and government.

Multiplex qPCR Assay Validation Characteristics

- PCR Efficiency
- Dynamic Range
- R^2 of calibration curve
- C_t variation at LOD

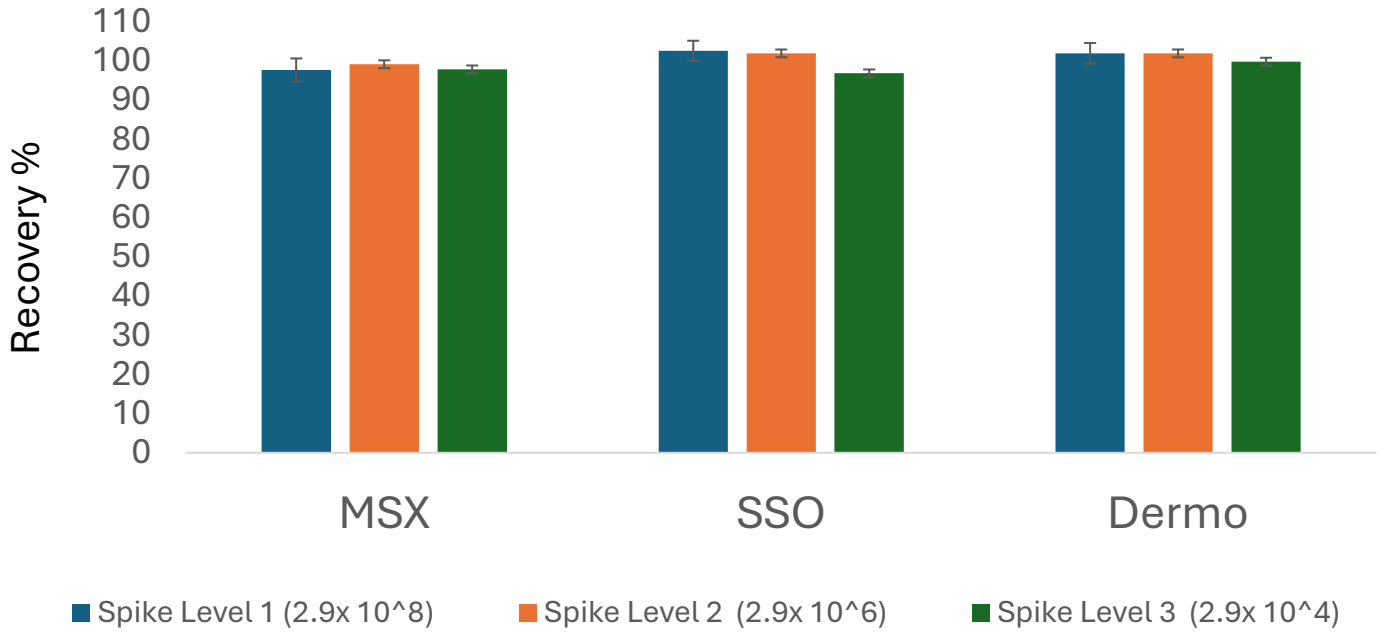
- Precision
- Accuracy
- Specificity: Inclusivity/Exclusivity
- Robustness
- Bias
- Detection limit
- Reporting Limit
- Uncertainty
- Expanded Uncertainty
- Proficiency test result (if available)

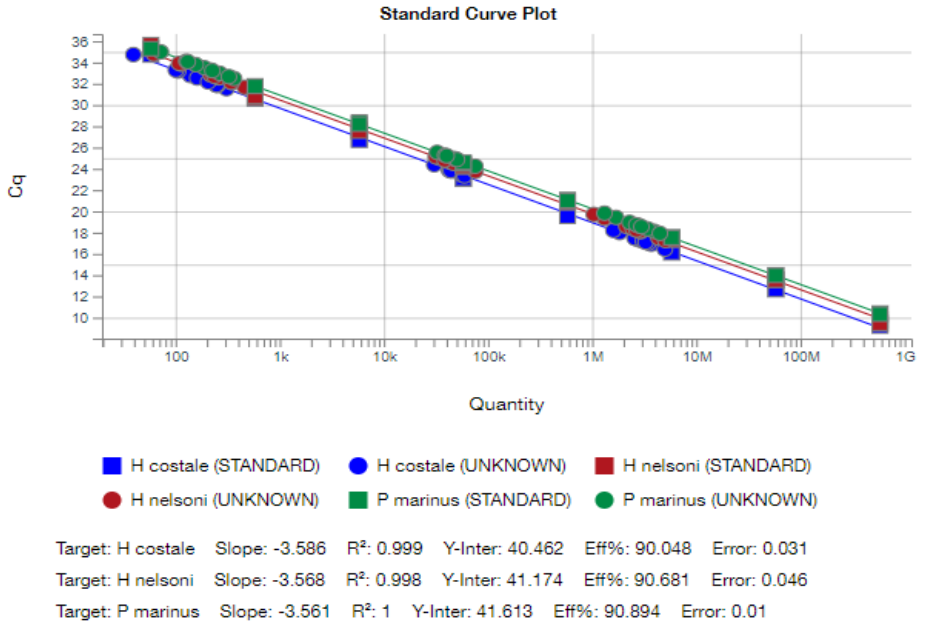
Multiplex qPCR Assay Limit of Detection



The assay limit of detection (LOD) was as low as 2.6 gene copies/µl for each target

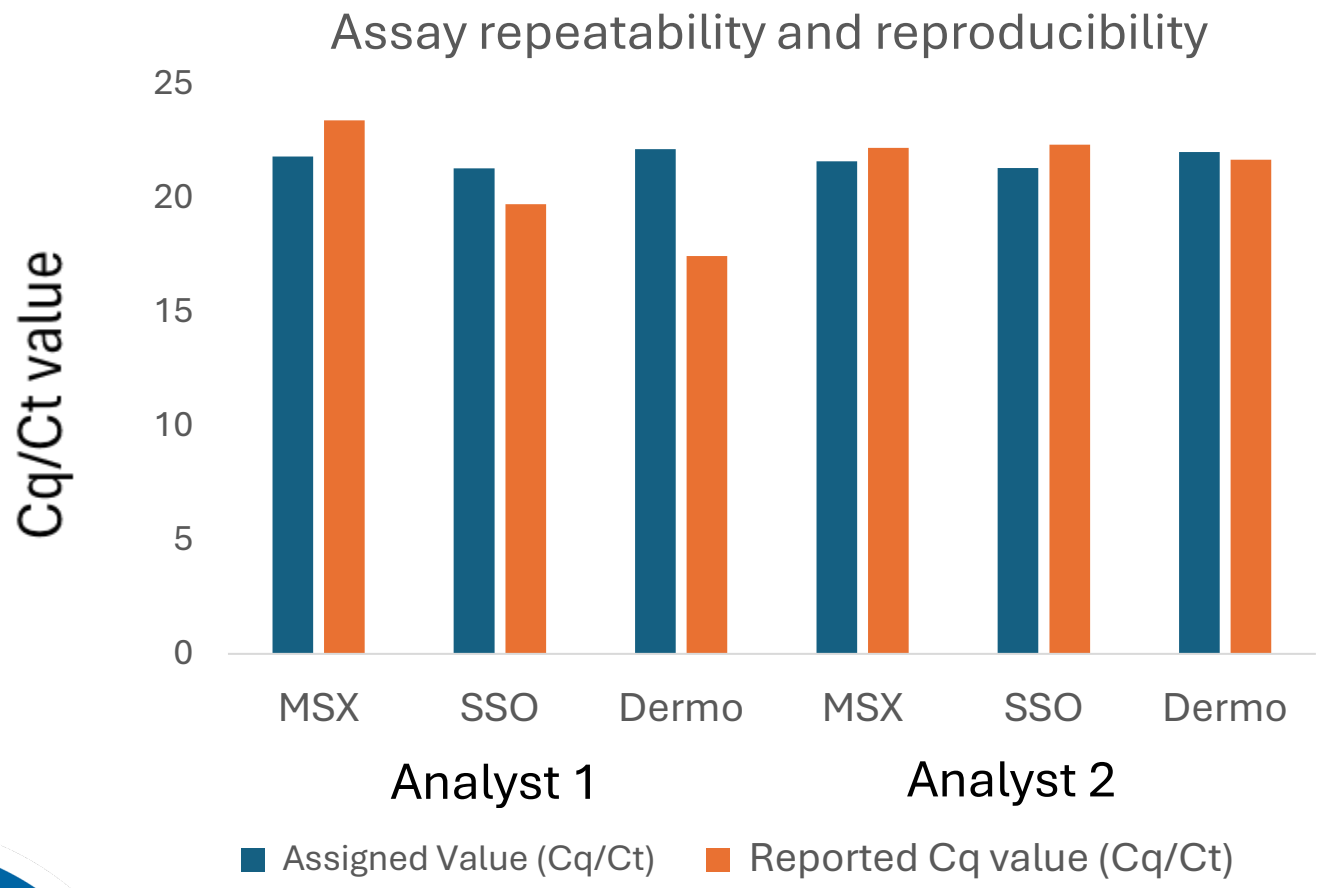
Accuracy of Multiplex qPCR





The results of artificial spikes exhibited accuracy and precision > 99%

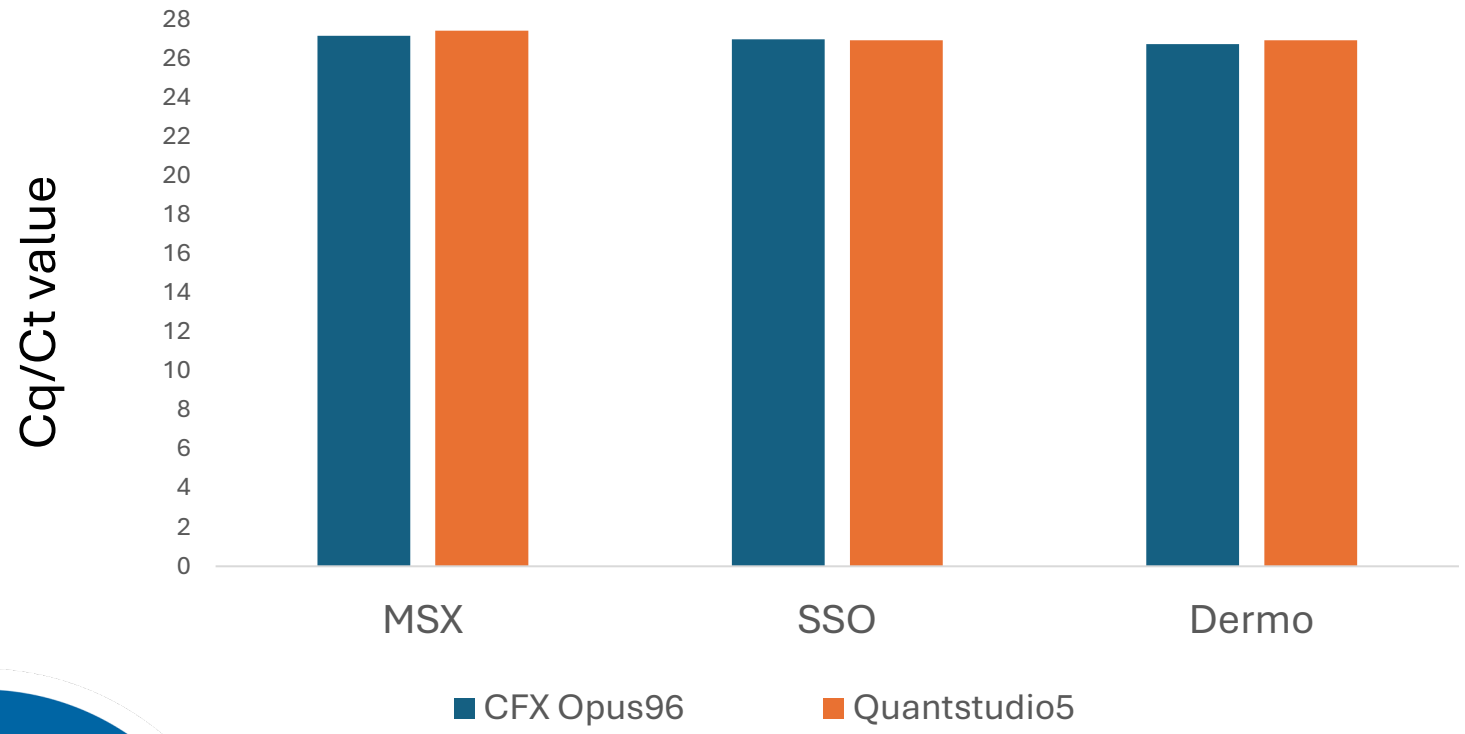
Blind Validation of Multiplex Assay



The results from two analysts were highly comparable and the repeatability and assay was in the range of 98-99%

Real-Time System Instrument Validation of Multiplex assay

n=10



CFX opus96 and Quant studio 5 two different qPCR instruments exhibited highly comparable results for three targets.

NBFF Project Results

Success!

- Multiplex qPCR Validation Completed.
- Added Mulitplex qPCR for MSX, SSO and Dermo to our scope of accreditation for ISO17025

DFO Funding Project

- *“ The development of rapid multiplex qPCR for the detection and quantification of three parasites MSX, SSO, Dermo of oysters (Crassostrea virginica) and MSX single-cell whole genome sequencing using long read sequencing platform.”*

MSX Science Research Funding

Description	Time frame	Funding amount
The development of rapid multiplex qPCR for the detection and quantification of three parasites (MSX, SSO, Dermo) of oysters (*Crassostrea virginica*) and MSX single-cell whole genome sequencing using long read sequencing platform Recipient: New Brunswick Research and Productivity Council (RPC) This project aims to enhance shellfish aquaculture industry disease diagnostic services by improving the methods for rapid detection of oyster parasites including: • *Haplosporidium nelsoni*, the causative agent of MSX (Multinucleate Sphere Unknown X) disease • *Haplosporidium costale*, the causative agent of SSO disease • *Perkinsus marinus*, the causative agent of Dermo The project also seeks to enhance our understanding of MSX virulence dynamics by using single-cell whole genome sequencing and routine surveillance in Atlantic waters to help inform hatchery production for disease-resistant oyster seed and MSX vaccine developers.	2024 to 2026	$256,562

https://www.dfo-mpo.gc.ca/science/aah-saa/msx-multinucleate-sphere-unknown-multinuclee-inconnue-eng.html#_03

Project Objectives

Objective#1

- A fully validated ISO 17025 accredited Multiplex qPCR assay of three Oyster parasites including:
  - *Haplosporidium nelsoni*, the causative agent of MSX (Multinucleate Sphere Unknown X) disease
  - *Haplosporidium costale*, the causative agent of SSO disease
  - *Perkinsus marinus*, the causative agent of Dermo

Nucleic acid Extraction platform for qPCR analysis

KingFisher™ Flex (96 deep-well format)

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Project Objectives

Objective#2

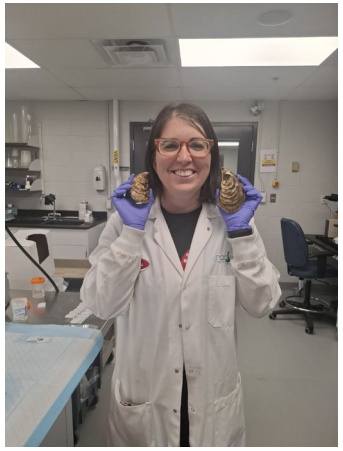
- MSX single cell sequencing
  - MSX virulence/resistance dynamics
  - Strain variability
  - Understanding of parasite survival mechanism, genetic variations and possible transmission routes etc.
  - Genomic epidemiology, assembly & annotation
  - Submission of MSX Genome to NCBI database

What is Challenging about MSX Sequencing

- Inability to culture *Haplosoridium nelsoni*
- Life cycle of the MSX parasite
- Genetic diversity within the MSX parasite population

Project Progress

- Worked with the Atlantic provinces to secure suspected high titer high incident of MSX oysters for tissue and hemolymph sampling.
- Simultaneous multiple approaches to capture a single cell of MSX.

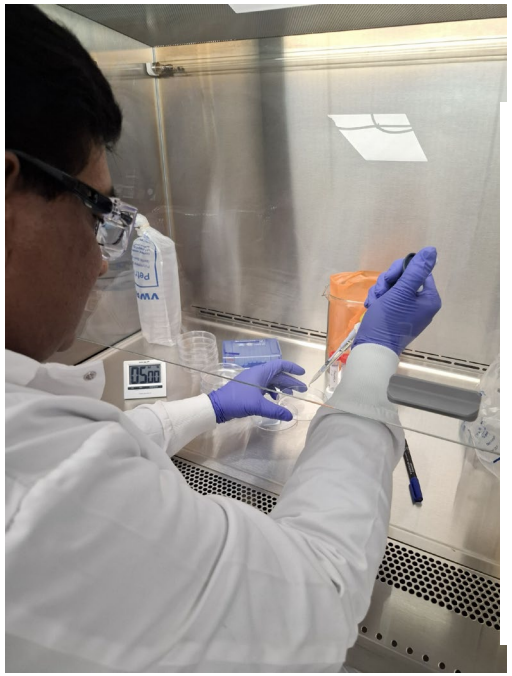


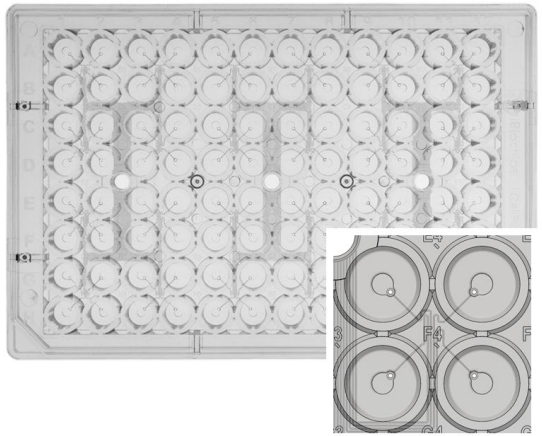
Project Progress

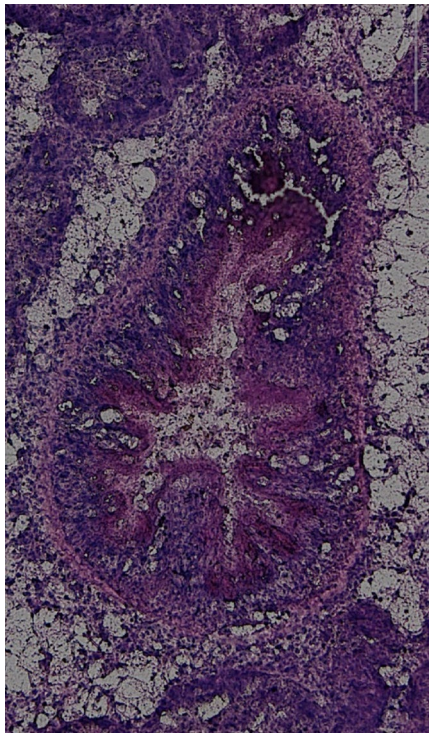
Our approaches have included :

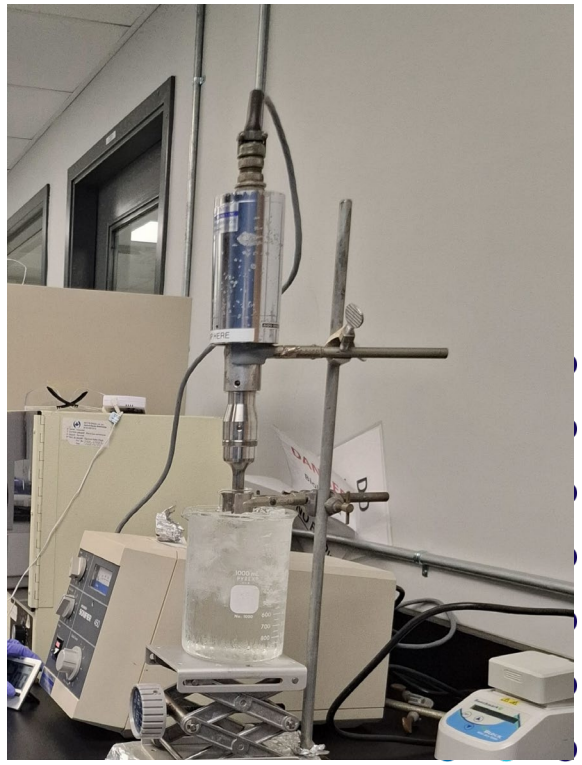
- Panning of hemolymph and archiving
- Microfluidics and single cell capture
- Laser Capture Microdissection
- Sonification of degraded tissue

Project Progress









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MSX Sequencing attempt

RPC sample ID	MSX Cq	Dermo Cq	SSO Cq
25-0005694-41-1	12.8	Not detected	Not detected
25-0005694-37-1	Control Not detected	Not detected	Not detected
25-0005694-72-1	16.6	Not detected	Not detected

Results

DNA pass all Quality checks

But unfortunately,

The prevalence of MSX

(~ 0.006 %).

Oyster DNA contamination

>99%

Here's the final LibQC results for Q048119: ALL PASS.

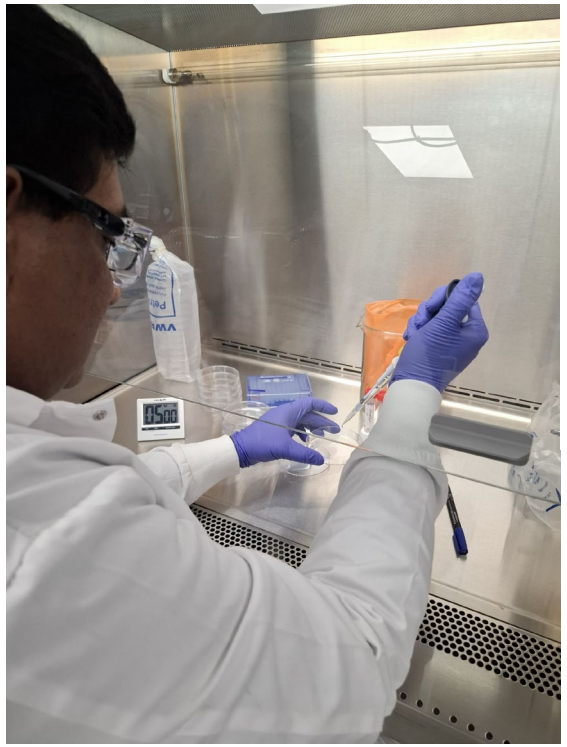
Name Sample	Concentration (qPCR in nM)
25-0005694-41-1	5.85
25-0005694-72-1	6.44
25-0005694-37-1	7.48

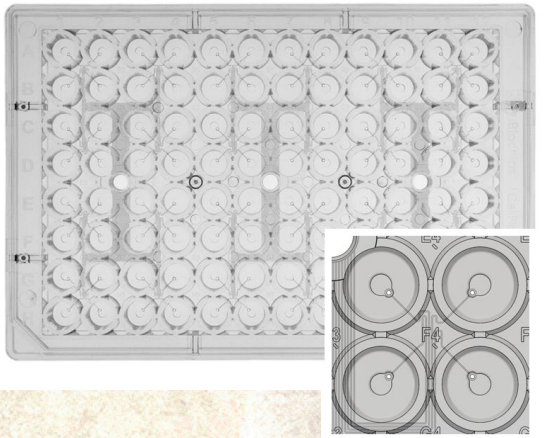
File	format	type	num_seqs
25-0005694-37/25-0005694-37-1_829672_S3_L001_R1_001.fasta	FASTA	DNA	1,377,048
25-0005694-37/25-0005694-37-1_829672_S3_L001_R2_001.fasta	FASTA	DNA	1,377,048
25-0005694-41/25-0005694-41-1_829670_S1_L001_R1_001.fasta	FASTA	DNA	1,387,974
25-0005694-41/25-0005694-41-1_829670_S1_L001_R2_001.fasta	FASTA	DNA	1,387,974
25-0005694-72/25-0005694-72-1_829671_S2_L001_R1_001.fasta	FASTA	DNA	1,470,755
25-0005694-72/25-0005694-72-1_829671_S2_L001_R2_001.fasta	FASTA	DNA	1,470,755

Trial, Error, Learning and Discovery

Project Progess

The panning technique followed by the microfluidic chip was the most hopeful approach.



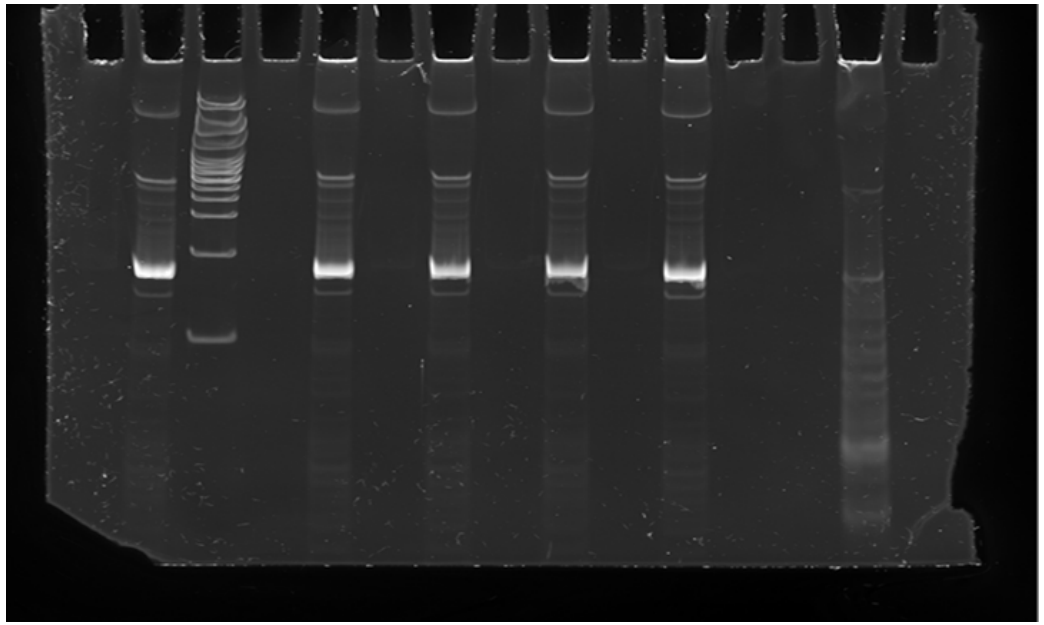


A Successful capture of a single cell!

Sample	MSX	SSO	Dermo	IAC
NEGH2O	UNDETERMINED	UNDETERMINED	UNDETERMINED	27.4
Project-1	UNDETERMINED	UNDETERMINED	UNDETERMINED	26.4
Project-1-dilute	UNDETERMINED	UNDETERMINED	UNDETERMINED	26.3
Project-3-4	8.9	UNDETERMINED	UNDETERMINED	26.3
Project-3-4-dilute	12.8	UNDETERMINED	UNDETERMINED	25.2
Project-A7	UNDETERMINED	UNDETERMINED	UNDETERMINED	26.5
Project-A7-dilute	UNDETERMINED	UNDETERMINED	UNDETERMINED	26.2
Project-Neg	UNDETERMINED	UNDETERMINED	UNDETERMINED	25.9
Project-Neg-dilute	UNDETERMINED	UNDETERMINED	UNDETERMINED	26.0

The Results

First run: DNA from our MSX captured microfluidic disc wells, displayed good band intensity.



Project Progress: Quality Control Checks

- Conventional PCR confirming % MSX, host contamination, bacterial contamination 16S
- Need to ensure good DNA integrity.
- Require >80% pure MSX DNA
- Send for whole genome long read sequencing

The Next Steps

- Continue to attempt to isolate pure single cells of MSX .
- Refine our current panning and microfluidics method.
- We are very close!

De Novo Assembly

Genomic DNA

Next-generation
DNA sequencing

...CATTCAGTAG...
...AGCCATTAG...
...GGTAGTTAG...
...GGTAGTTAG...
...AGCCATTAG...
...GGTAAACTAG...

Millions-billions of ***reads***
~30-1,000 nucleotides

RESEQUENCING

Align reads to ***reference genome***
and identify variants

De Novo ASSEMBLY

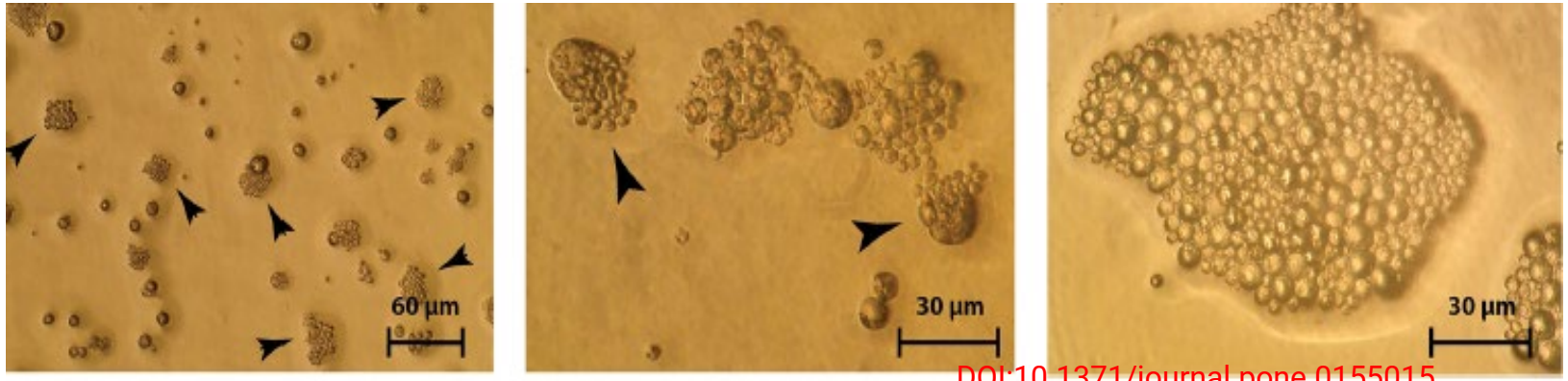
Construct ***genome sequence***
from overlaps between reads

Genomic characterization of *Perkinsus marinus* (Dermo disease) in Atlantic Canadian Oysters

*This project is funded, in part, by **Genomic Atlantic** under the Genomics Opportunity Review Program (GORP), the governments of NS, NL, PEI and NB, and the Government of Canada via ACOA*

Project Main Objectives:

- Generate high-quality, assembled reference genomes for Dermo.
- Comparative genomic analyses to investigate virulence factors and transmission dynamics.
- Deposit genome to public database i.e. NCBI-GenBank



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RPC MSX Project Team

Thank You!

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