CAN THIRD GENERATION SEQUENCING RAPIDLY DETECT HARMFUL ALGAL BLOOMS(HABs) ?

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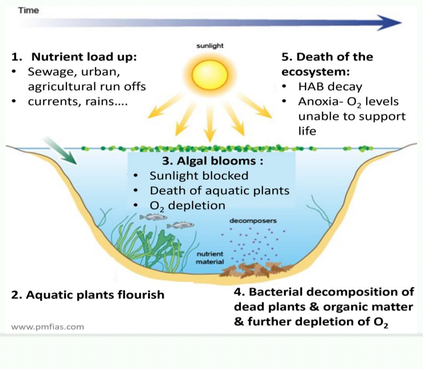
INTRODUCTION

Phytoplankton cause algal blooms, some of which are harmful - HABs



Massive amounts of fish kills due to past events of HABs in Singapore

Dinoflagellate blooms

- 2009- 200,000 farmed fishes
- 2014- 500 **tonnes** of fish from 53 farms
- 2015 - 600 **tonnes** of fish in 77 farms/ **loss at $1.3 million/farmer**

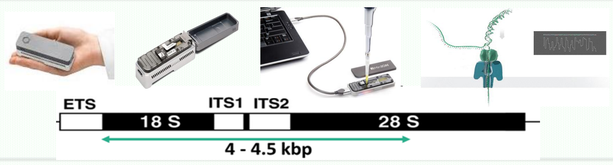
- **HAB mitigation & control**: Early & precise characterization of bloom causing organisms.
- **Challenge**: **Tedious conventional methods** requiring expertise and slow analysis.
- **Solution: Third Generation Sequencing with MinION™** - relatively lower costs, precise & rapid analyses.

OBJECTIVES

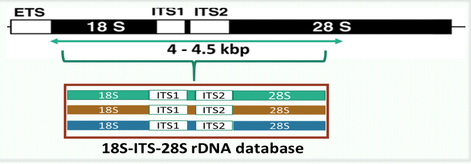
1. Design and optimize:

I. Methodology for MinION sequencing of eukaryotic microalgal rDNA unit (18S - 28S)

II. Bioinformatics pipeline for error correction and analyses of sequence



2. Build a reference database containing these rDNA error corrected sequences



SEQUENCED SAMPLES

Diatoms	Dinoflagellates	Chlorophytes
Coscinodiscus sp.	*Scrippsiella* sp.	*Tetraselmis* sp.
Cyclotella sp.	*Karenia mikimotoi*	*Ostreococcus tauri*
Phaeodactylum tricornutum	*Karlodinium cf veneficum*	*Picochlorum sp.SENEW3*

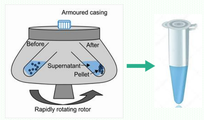
EXPERIMENTAL METHODS

1 Sample collection, filtration and preparation

Environmental samples

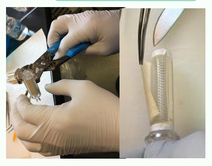
1L filtered through 0.22µm sterivex

Single species

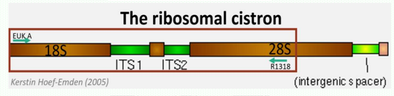


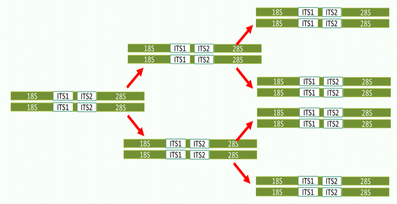
20-30mL of cultures
500g for 10mins

2 DNA extraction

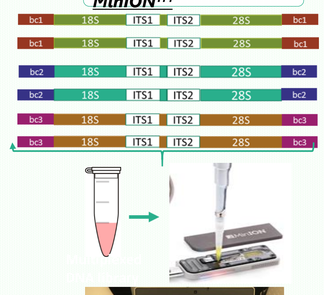


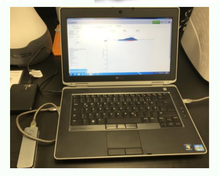
3 PCR to amplify rDNA cistron





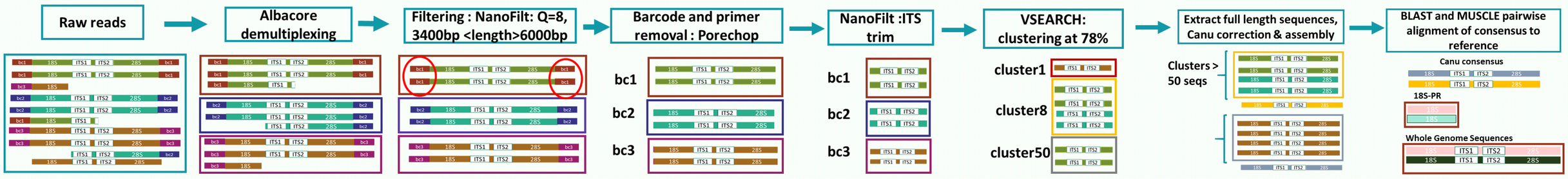
4 Barcoding, multiplexing and Amplicon sequencing using *MinION™*





RESULTS

BIOINFORMATICS PIPELINE –ERROR COORECTION AND READS ANALYSES



Single species sequences analyses

Table 1: Similarity of canu (MInION) sequences compared to PR² references (containing only 18S sequences) based on the pairwise distance matrix using MEGA version 7.2

	Coscinodiscus sp.	*Cyclotella* sp.	*Scripsiella* sp.	*Karlodinium veneficum*	*Karenia mikimotoi*	*Tetraselmi* sp.
Accuracy of consensus compared to PR2	99.50%	98.95%	99.70%	99.50%	99.00%	99.30%

Table 2: Similarity of canu (MInION) sequences compared to NCBI-WGS references (full length rDNA sequences) based on the pairwise distance matrix using MEGA version 7.2

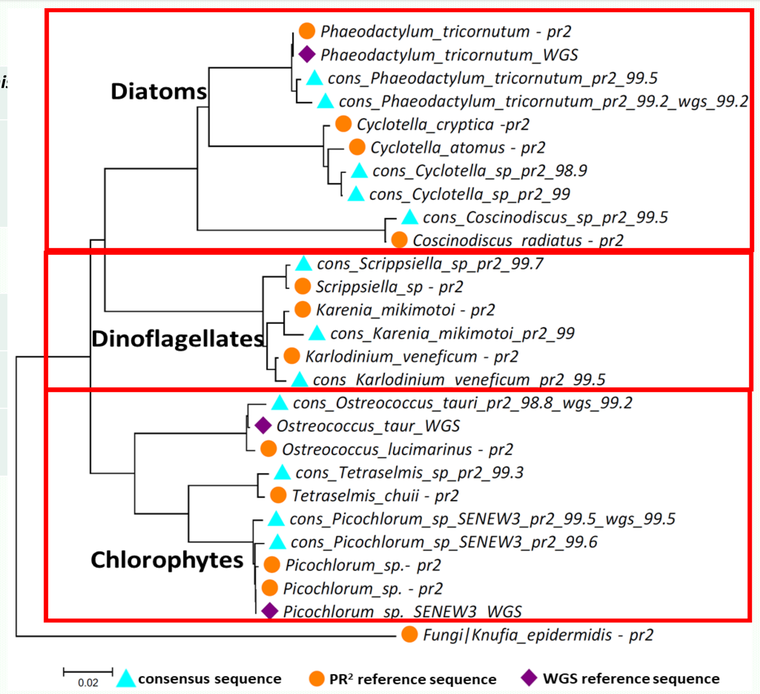
	Picochlorum sp SENEW3	*Ostreococcus tauri*	*Phaeodactylum tricornutum*
Accuracy of consensus compared to PR2	99.55%	98.80%	99.20%
Accuracy of consensus compared to WGS	99.50%	99.20%	99.20%

Table 3: Reduction in error rates and increased Q-scores of MinION

Single species sequences	Sequencing error rate	Q-score
Raw reads	**12 – 22%**	6.6 - 9
Error corrected reads	**≤ 0.7%**	21.5

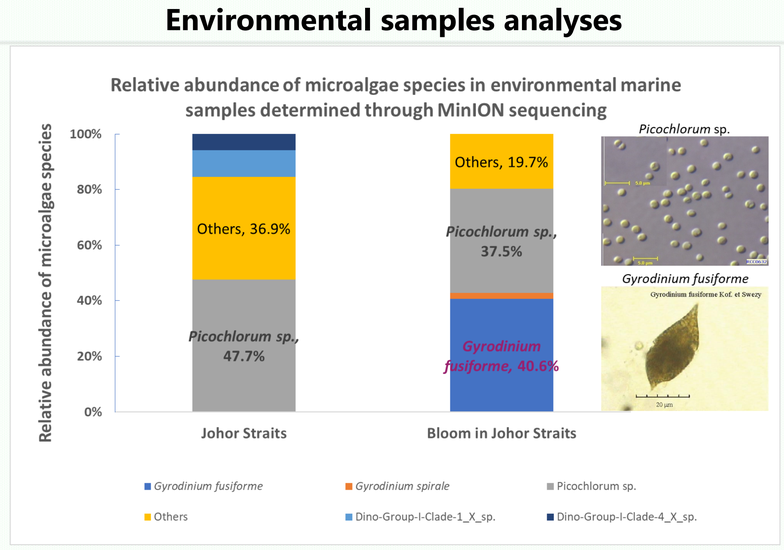
Q (Phred score) = -10 $\log_{10}$ P

Average accuracy of the reads = 99.3%

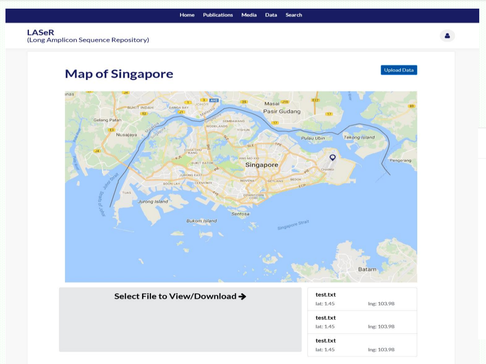


Phylogenetic tree constructed using the Neighbor Joining method of canu consensus sequences clustered with PR2 and WGS reference sequences

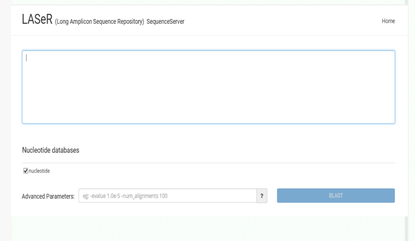
Environmental samples analyses



'LASeR ' :Long Amplicon Sequence Repository- error corrected rDNA sequences



- Deposit references
- Search & download refernces



Microscopy Vs MinION™

Factor	Microscope	MinION™
Analysis time	~5-48hrs. per sample	~<2 hrs. for 96 samples/MinION
Identification level	Phylum, genus and sometimes species level.	Phylum, genus and species level identification
Detection limit	2 cells/mL	<10 cells/mL
Accuracy	Dependent on taxonomic expertise	Taxonomist not required due to the availability of a bioinformatics pipeline

CONCLUSION & FUTURE DIRECTIONS

***MinION™* sequencing technology - a great potential HABs monitoring- RAPID & PRECISE DETECTION**

- Portable sequencer for field applications enabling rapid taxonomic characterization.
- Increase error correction iterations with Canu for read quality improvement for clustering at species and subspecies level.
- Series dilution and qPCR for quantification with MinION.
- Target toxin genes in phytoplankton through the CRISPR-CAS9 targeting mechanism.

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