Uncover bacterial pathogen identity using Nanopore MinION

A collaboration between WorldFish, The University of Queensland, WilderLab, Centex and GeneSEQ

Online virtual workshop 9th of August 2021

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

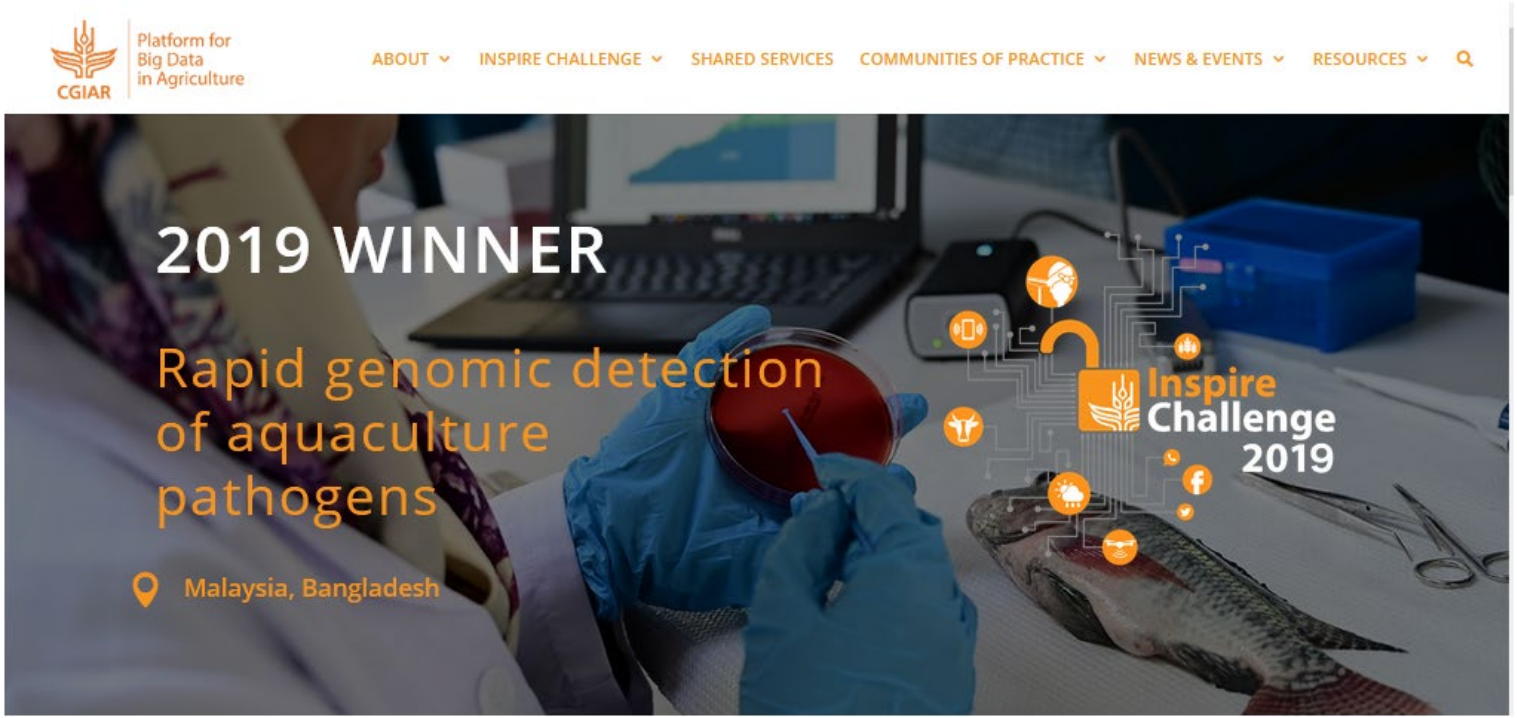
WILDERLAB

MAHIDOL UNIVERSITY BIOTEC-NSTDA

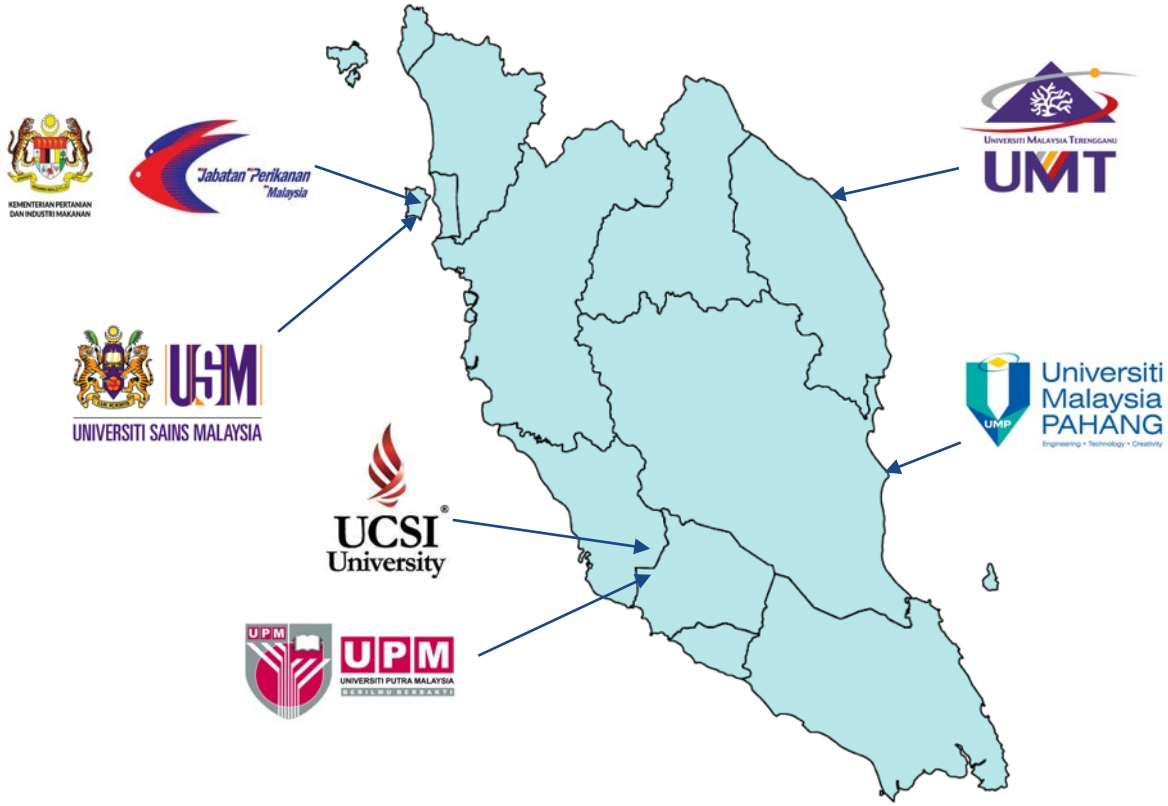
GeneSEQ NEXT GENERATION SEQUENCING

WorldFish

Inspire Challenge project

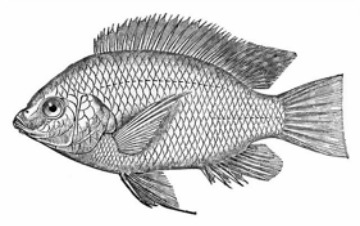


Malaysian institutions and participants



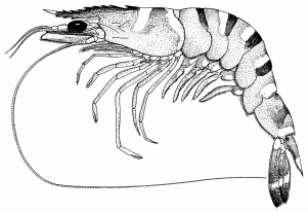
Hosts & pathogens from Malaysia

Oreochromis sp.



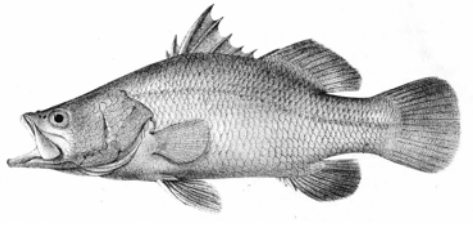
Streptococcus agalactiae

Penaeus monodon



Vibrio parahaemolyticus / V. owensii / V. harveyii

Lates calcarifer



Vibrio parahaemolyticus

Seawater

Photobacterium sp. / *Bacillus* sp.
Vibrio sagamiensis

Bioflocs

Enterobacter sp.

Holothuria leucospilota

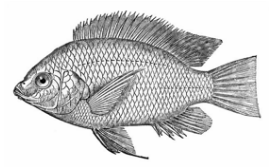
unknown

Sediment

Bacillus cereus

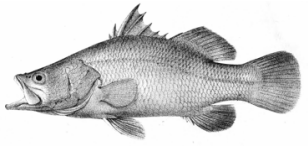
Hosts & pathogens from other parts of the world

Tilapia
Oreochromis sp.



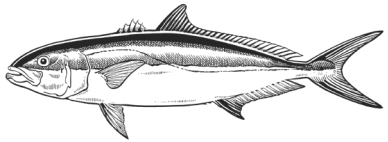
Streptococcus sp; Edwardsiella sp.; Aeromonas sp; Vibrio sp.; Infectious Spleen and Kidney Necrosis Virus (ISKNV)*;* Tilapia lake virus (TiLV)

Barramundi
Lates calcarifer



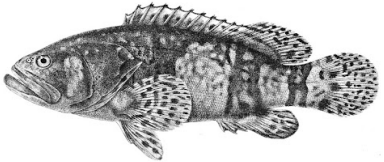
Vibrio sp; Streptococcus iniae

Kingfish
Seriola lalandi



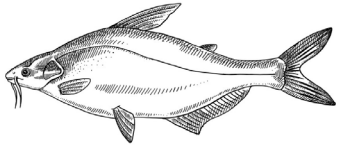
Photobacterium damselae

Qld giant grouper
Epinephelus lanceolatus



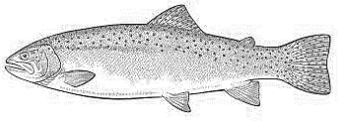
Streptococcus agalactiae

Pangasius
Pangasius sp.



Aeromonas hydrophila

Rainbow trout
Oncorhynchus mykiss

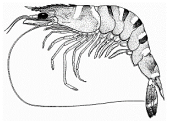


S. iniae; Yersinia ruckeri

Atlantic salmon
Salmo salar

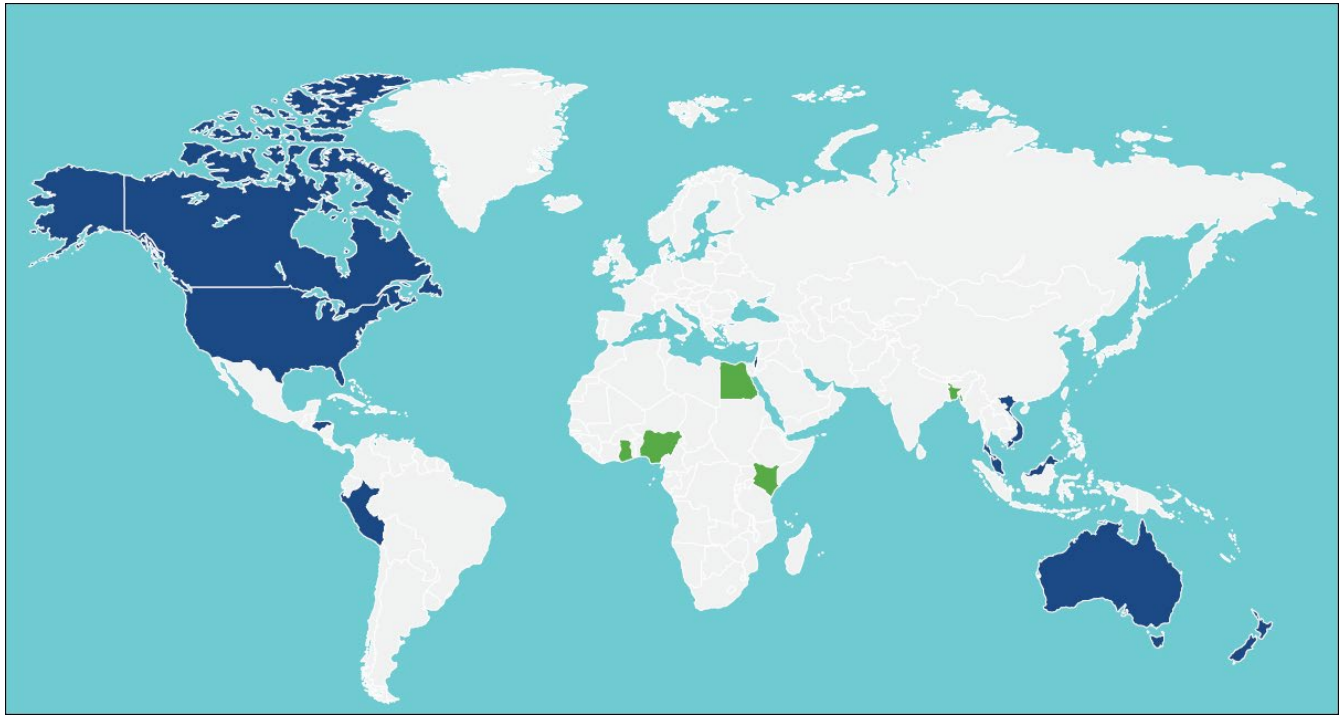
Y. ruckeri; Tenacibaculum maritimum

Shrimps
Penaeus monodon/vannamei



Vibrio sp; *Aeromonas* sp.

Origins of the pathogens



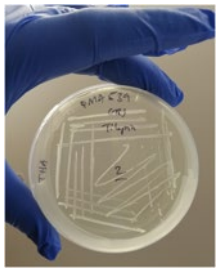
Aquaculture pathogens sequenced in this project

Honduras, Ecuador, Peru, USA, Canada, Israel, Australia, New-Zealand, Vietnam, Thailand, Malaysia

Future works using Nanopore

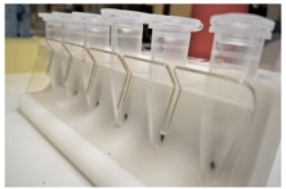
Bangladesh, Egypt, Ghana, Nigeria and Kenya

Bacterial samples DNA extraction and Illumina sequencing



Participants bacterial isolates

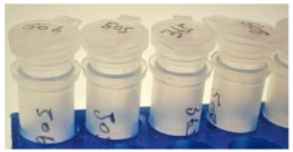
bacterial suspension inactivated in 100% ethanol

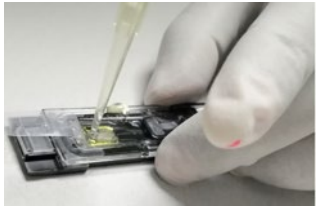


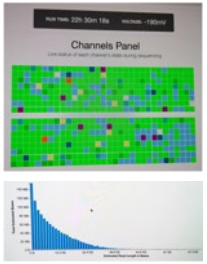
DNA extraction/ quantification

Illumina sequencing (short reads)

DNA samples for Nanopore sequencing







Raw Nanopore and Illumina data combined for analysis

Today’s agenda

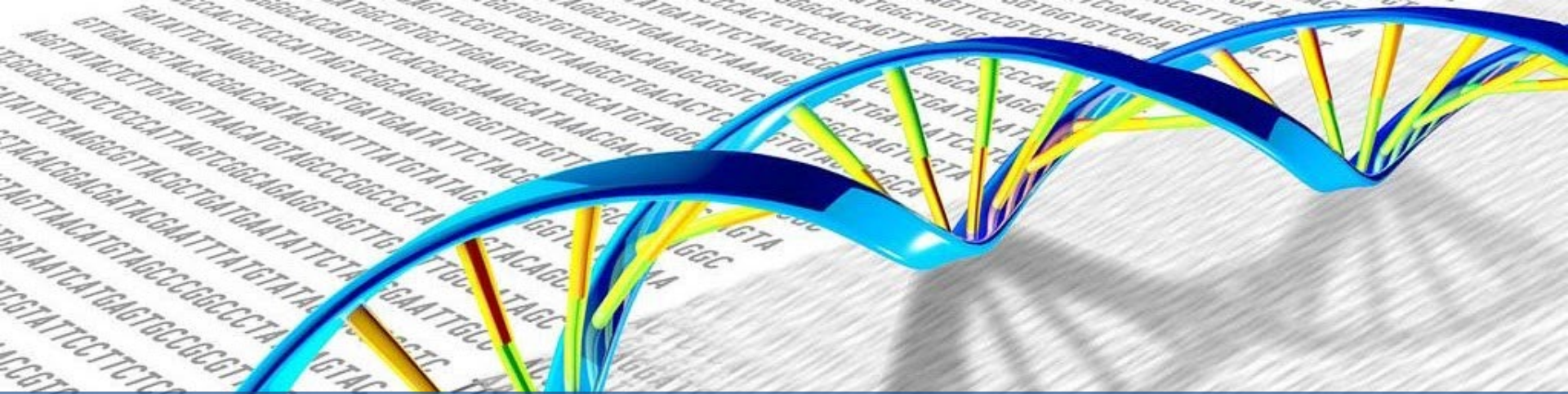
Part 1
- Sequencing technologies from Sanger to Nanopore
- Applications

Part 2
- Sample collection & DNA extraction

Part 3
- DNA library prep for Nanopore sequencing

Part 4
- Bioinformatics analyses

Part 5
- Participant Data Overview



From Sanger to Nanopore sequencing technologies

Associate Prof Andrew Barnes

The University of Queensland

First generation sequencing: Sanger technology

	Reagents	Reaction	Products	Capillary electrophoresis	Performance
First-generation sequencing / Sanger technology	Primers Template + dNTPs Enzyme + Dye-labeled terminators A C G T	Annealing, Extension	A, AT, ATT, ATTC, ATTCC, ATTCCA, ATTCCAC		Applied Biosystems® 3500 Series Genetic Analyzers Read length up to 850 bp Maximum throughput 138,000–414,000 bp/day Run time at least 30 min

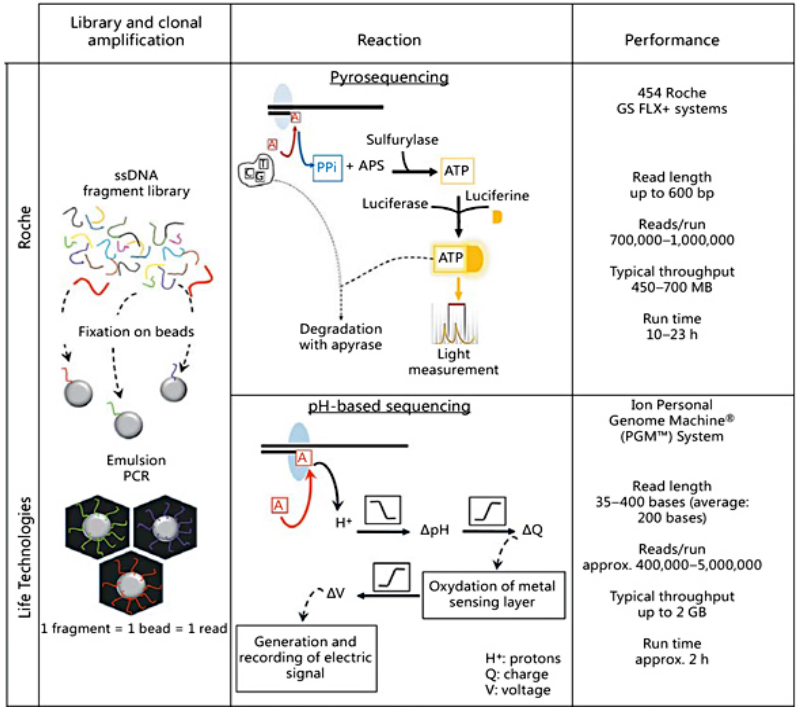
https://doi.org/10.1159/000477808

2G NGS platforms: Life Technologies Roche

NGS: Next Generation Sequencing

http://www.marbigen.org/content/flx-454-sequencer

https://tools.thermofisher.com/content/sfs/brochures/PGM-Specification-Sheet.pdf



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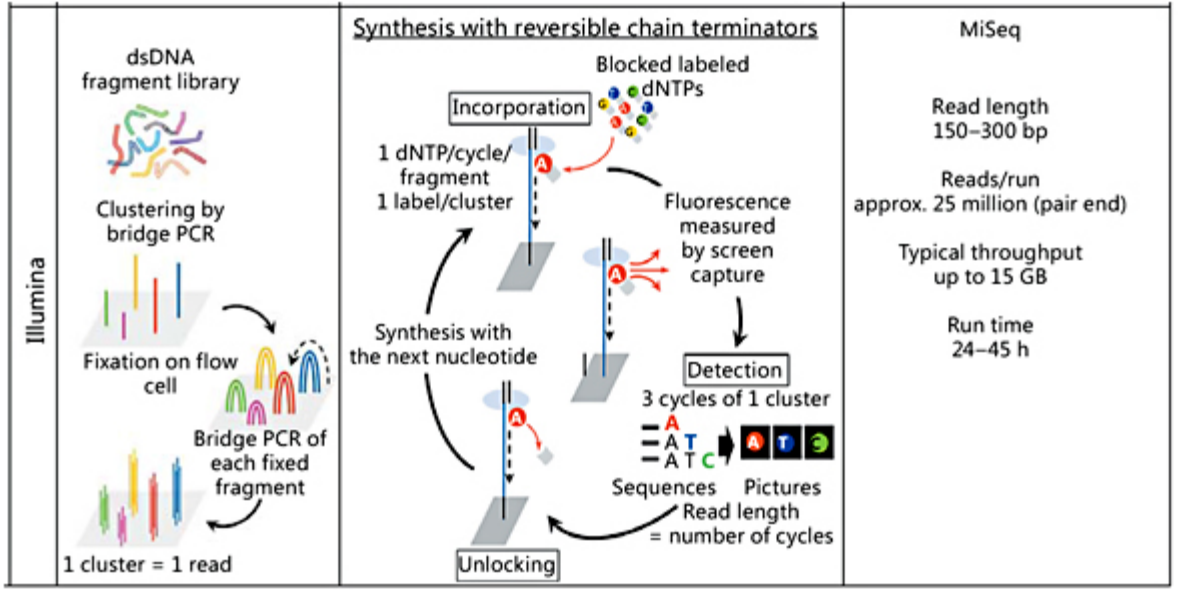
2G NGS platforms: Illumina



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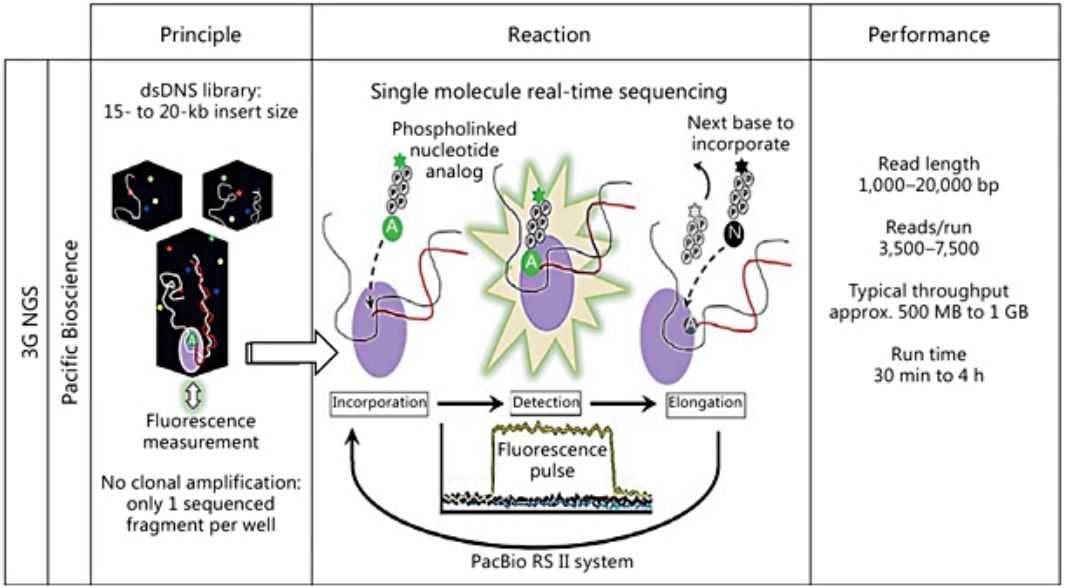
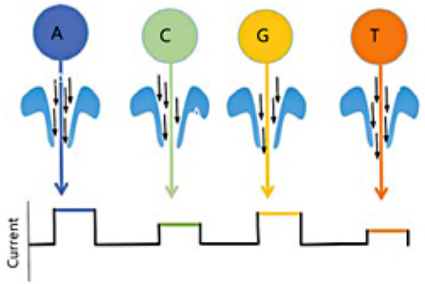
3G NGS Pacific Bioscience

https://www.wikiwand.com/en/Pacific_Biosciences



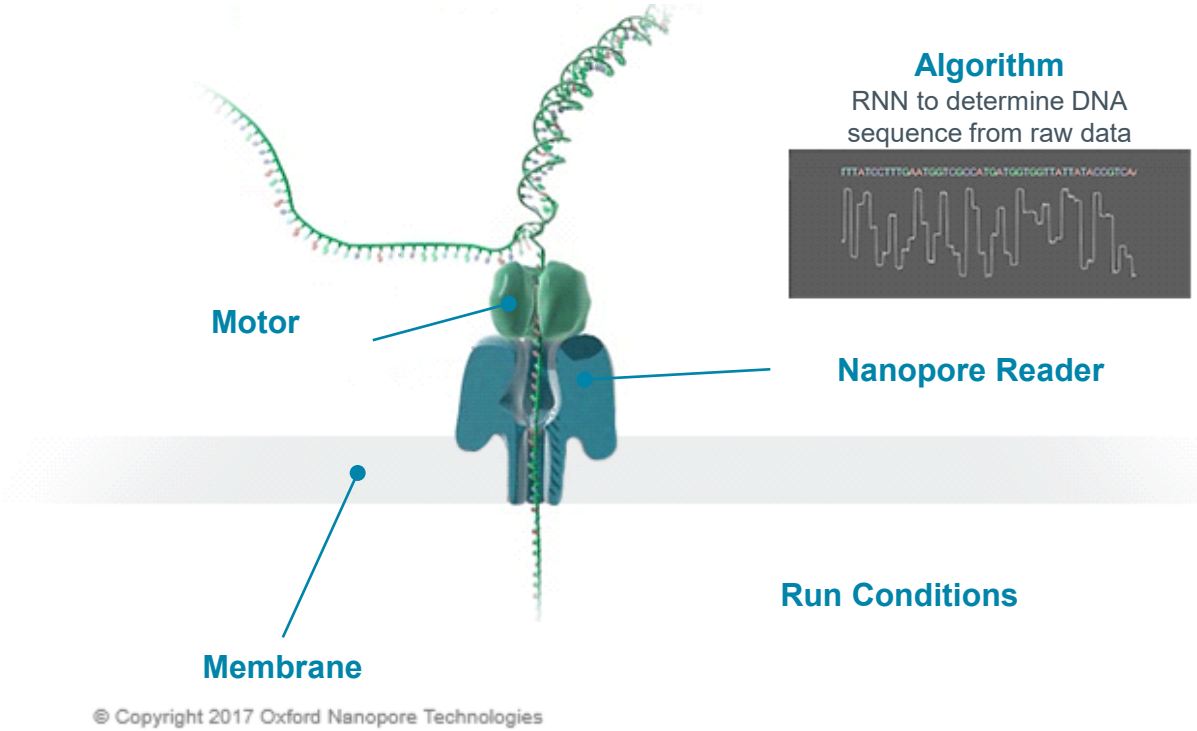
https://doi.org/10.1159/000477808

3G NGS Oxford Nanopore

		Principle	Reaction	Performance
4G NGS	Oxford Nanopore	dsDNS library: 15- to 20-kb insert size  No clonal amplification A single molecule per nanopore per well	Single molecule real-time sequencing  Nucleotides pass through the pores and modify the ionic current Base calling Measurement of the modulation	MinION Read length 5,000–200,000 bp Reads/run >1,000,000 reads Typical throughput >1 GB Run time depends on the input speed: 1 bp/ns Weight 90 g

https://doi.org/10.1159/000477808

Nanopore DNA/RNA sequencing - how does it work?



Algorithm

RNN to determine DNA sequence from raw data

Nanopore Reader

Run Conditions

Multiple nanopore sensors arrayed in one device sequencing in parallel

Operate independently but at the same time

Application of NGS in aquaculture

Fish farmers' questions during disease outbreak

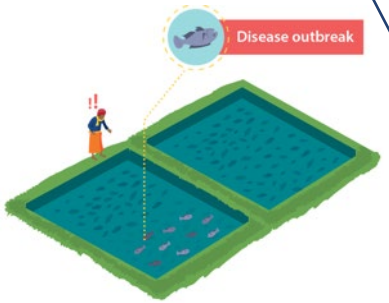
What is killing my fish?

How can I treat them?

How can I prevent disease in the next crop?

Genome-based diagnosis of pathogens in aquaculture

NGS
(MinION/Illumina)

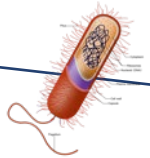


Pathogen Species ID and epidemiology (MLST)

Virulence factor identification

Antibiotic susceptibility

Molecular serotyping
(vaccine formulation)



Management of disease outbreak at farm level

Sample collection & DNA extraction

Dr Jerome Delamare-Debouttteville

WorldFish

Sampling materials for fish bacteriology

Anesthetic (e.g. MS-222, benzocaine, clove oil).

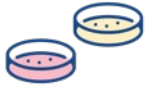
Spray or squirt bottle filled with suitable disinfectant (e.g. normal grade 70% ethanol).

Sterile single-use loops, plain cotton swabs or reusable metal loops.

Plastic beaker (50–100 mL) to disinfect instruments in 70% ethanol.

Post-mortem sheet, clean tissue paper or aluminium fold to create a clean surface to sample the fish.

Sterile dissection kit (scissors, scalpel, forceps, tweezers etc.).



Trypticase Soy Agar (TSA) plates (or any other bacteriological agar as per study requirement).

Box, transport container, strong resealable plastic bags to pack and transport samples.

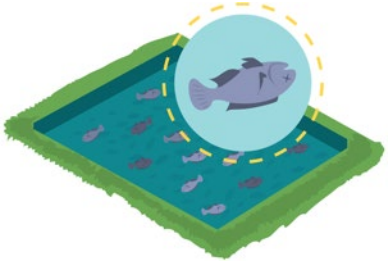
Paper towels (enough for each fish and to clean surfaces and instruments).

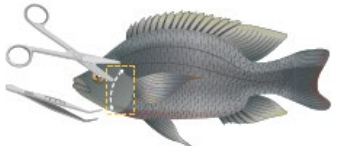
Portable Bunsen burner to create a sterile environment around the sampling area and to sterilize dissection instruments between fish.

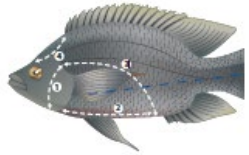
Bacteriological transport swabs (if no TSA plates to be used for field sampling).

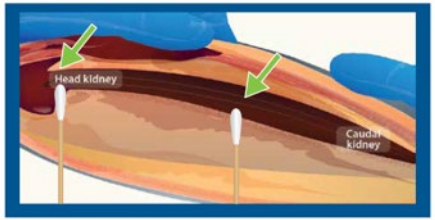
Plastic biohazard clinical bags for waste disposal.

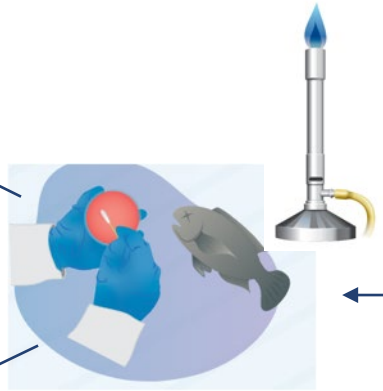
Bacteriology sampling from diseased fish

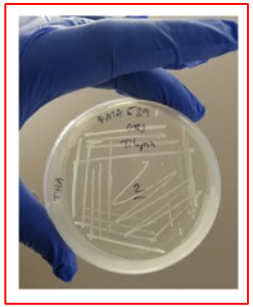












DNA extraction

pure bacterial suspension

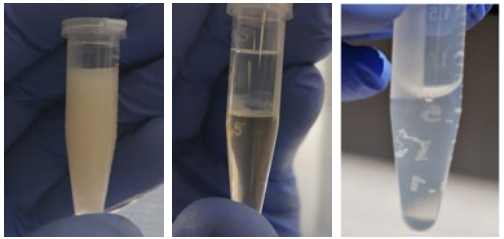
BIOBANKING

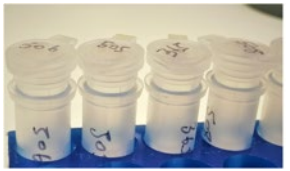
Cells lysis methods

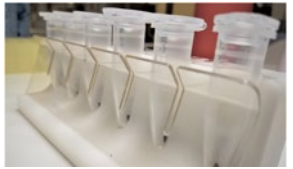
	Advantages	Disadvantages
SDS+Proteinase K	Overall good performer for DNA extraction	May lead to co-precipitation of carbohydrate
CTAB	Sample with high polysaccharide (e.g. capsulated/mucoid microbe)	CTAB is detrimental to environmental
Lysozyme pretreatment +SDS	Suitable for gram positive microbe	May not work across all gram positive microbe
Mechanical disruption (Bead beating)	Samples with tough/thick cell wall	Can lead to fragmentation of DNA (higher smearing)

DNA purification methods

	Advantages	Disadvantages
Phase separation (chloroform extraction)	High DNA yield and integrity Cheap	Requires equipment, generates toxic chemicals (chloroform and phenol), relatively time consuming
Column-based separation	Fast and convenient.	Requires equipment, expensive, often low-yield and molecular weight and require multiple centrifuge steps. Not scalable
Magnetic silica/ carboxylated beads	Scalable, fast and convenient, only magnet needed, high DNA yield and integrity	High cost of commercially produced beads





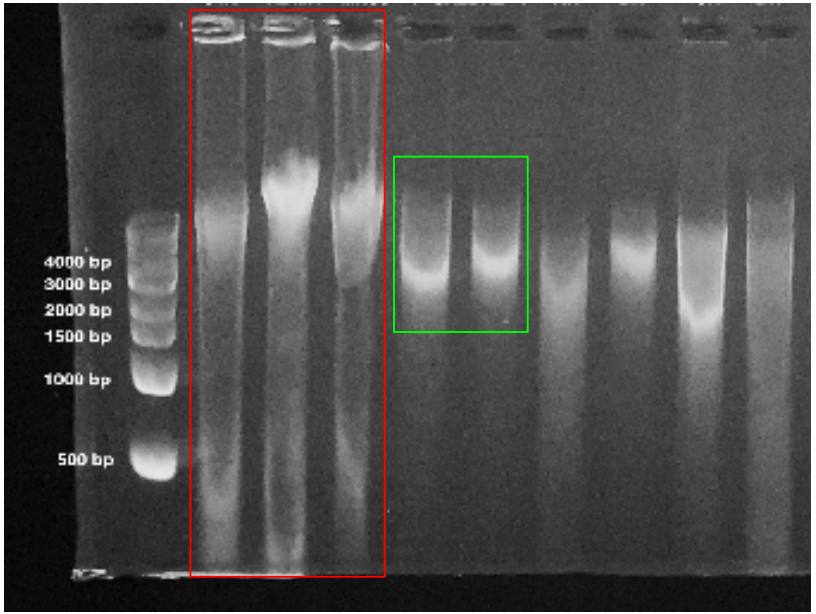


DNA concentration

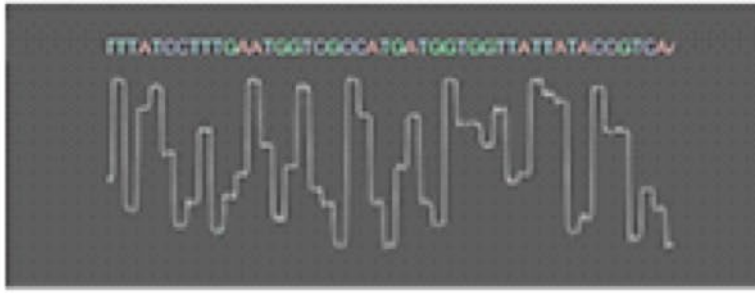
Qubit Fluorometer. Measure the concentration of dsDNA based on the fluorescence emitted by proprietary dsDNA-specific binding dye.

DNA quality control

Nanodrop spectrophotometer to estimate DNA purity based on absorbance measurement.



DNA integrity assessed by agarose gel electrophoresis.



DNA library prep for nanopore sequencing

Dr Jerome Delamare-Debouteville

WorldFish

The University Of Queensland AUSTRALIA

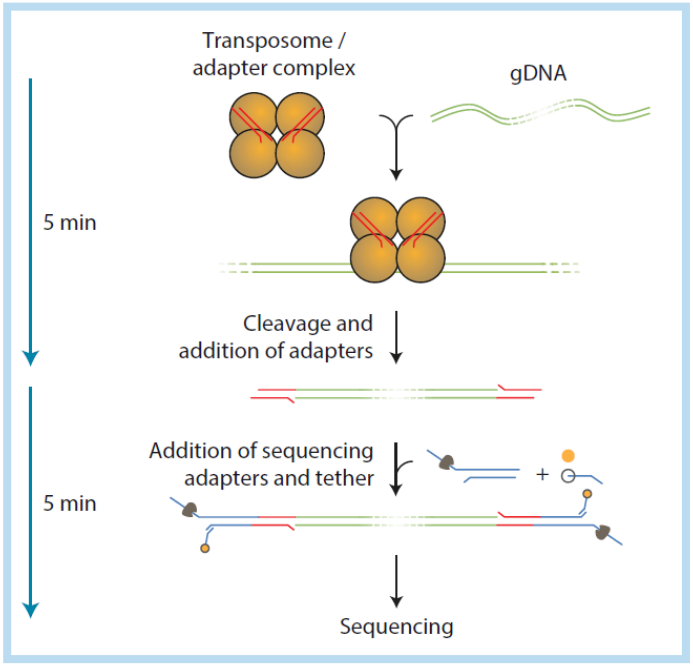
WILDERLAB

Mahidol University BIOTEC-NSTDA

GeneSEQ NEXT GENERATION SEQUENCING

WorldFish

Library Preparation: Transposase Approach (Rapid Kit)

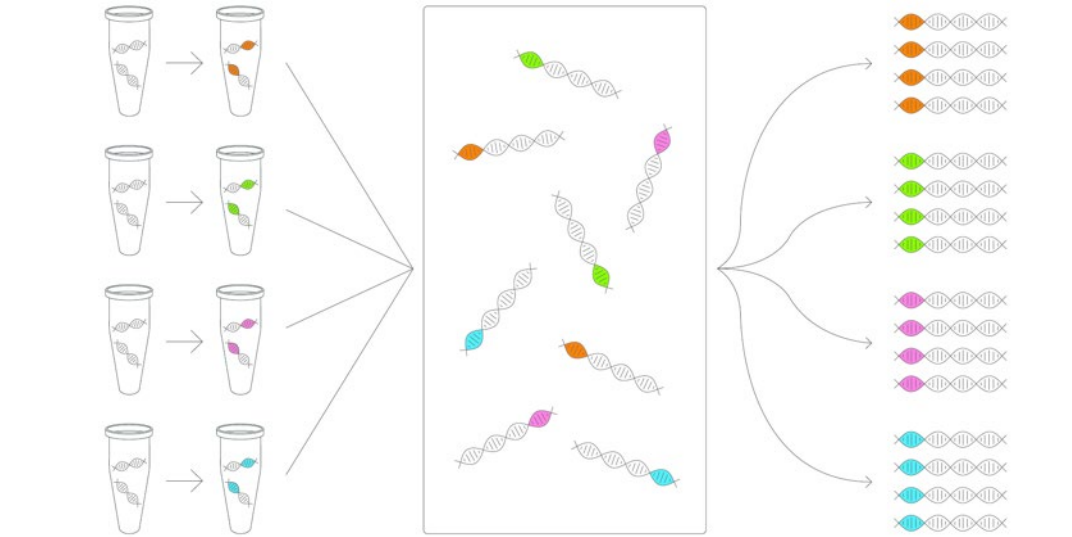


- Very rapid library generation with minimum laboratory requirement
- Fragmentation: tranposase based
- Input amount: 400 ng HMW gDNA
- Multiplexing options
- Can generate several Gbases in a run (~3-5Gb)

Barcoding - Multiple Sample in One Nanopore Run

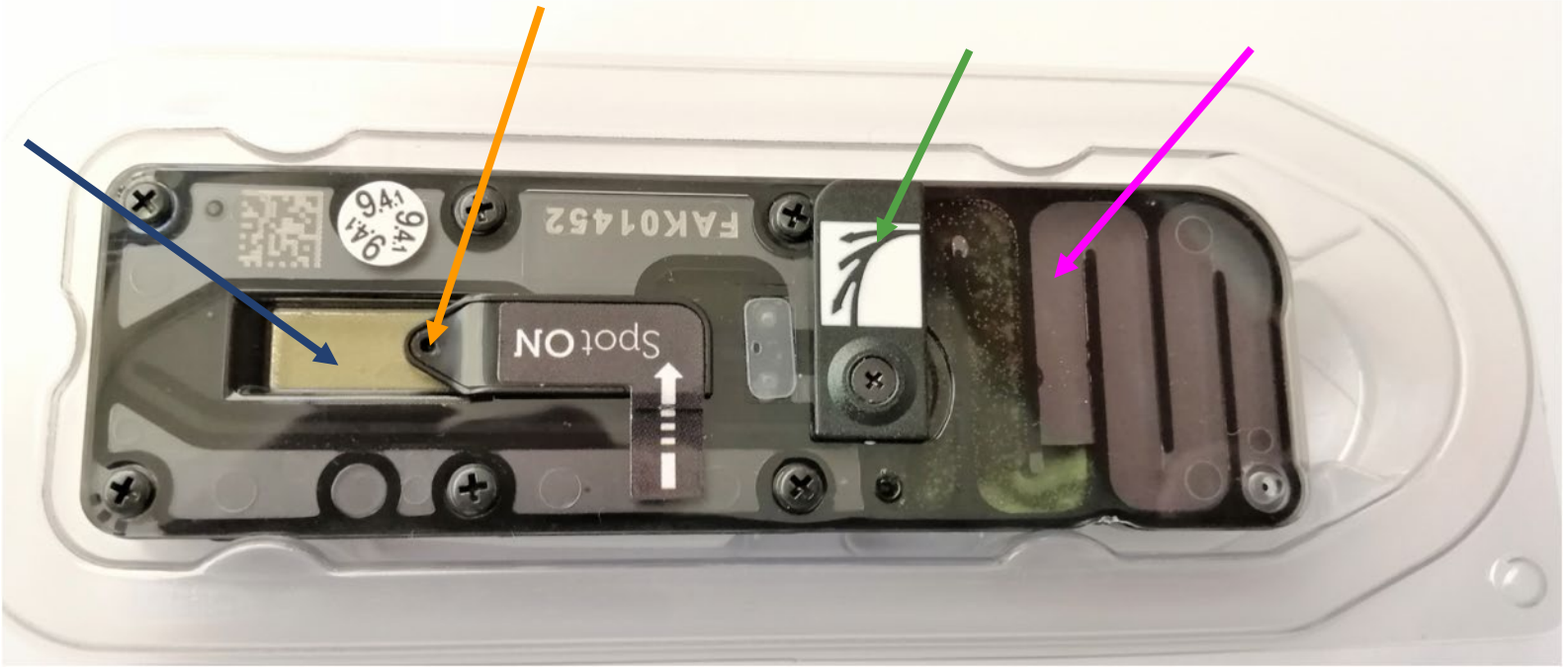
Kits are available to multiplex several samples, to maximise flow cell usage.

Up to 96 barcodes are supplied in Oxford Nanopore kits.

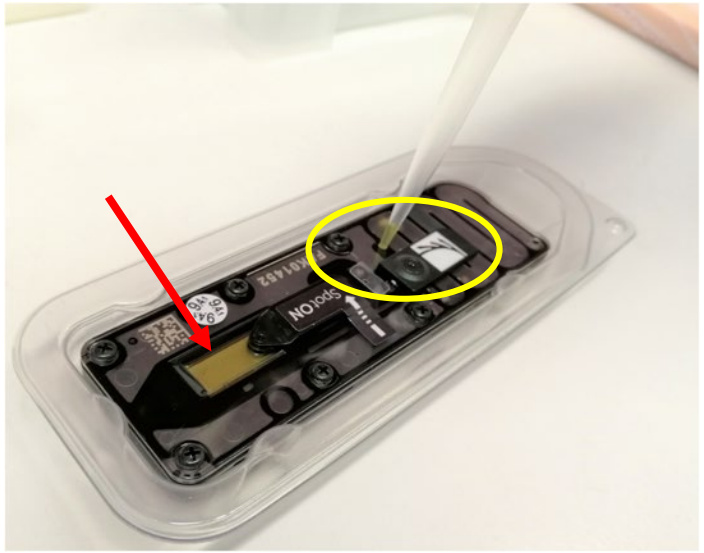


Barcode multiple samples → Pool and sequence → Separate and analyse

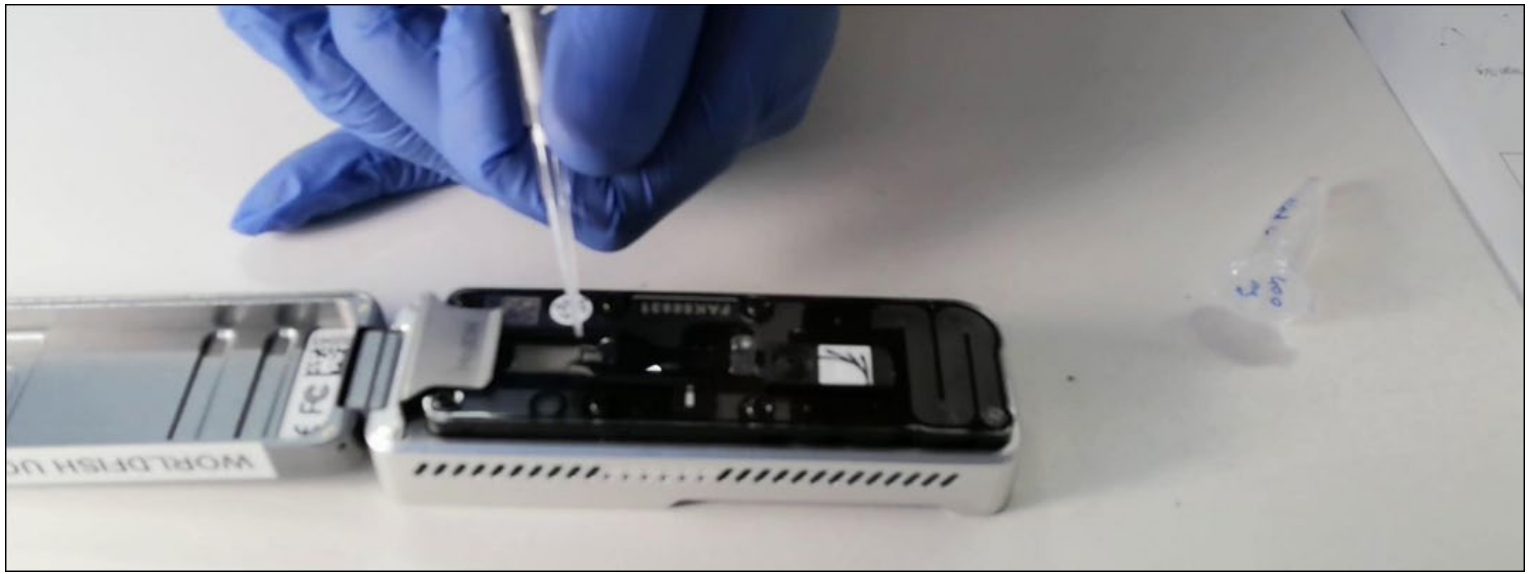
A functional MinION Flowcell for Sequencing



Flow cell Priming

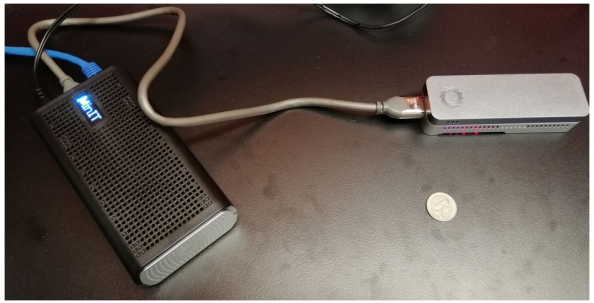


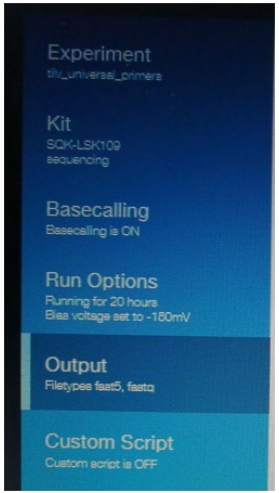
Library loading on Minion flow cell

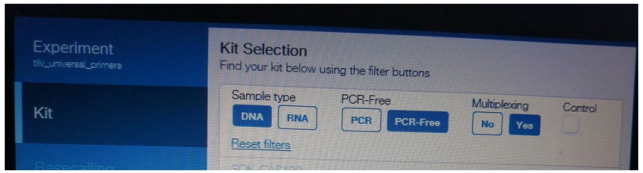


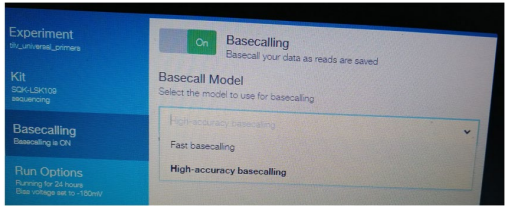
Library loaded

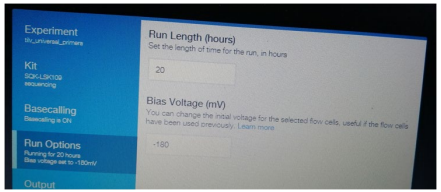
Starting a sequencing run

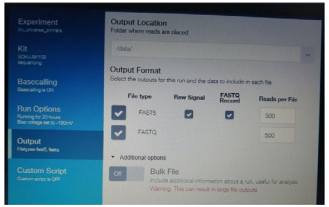




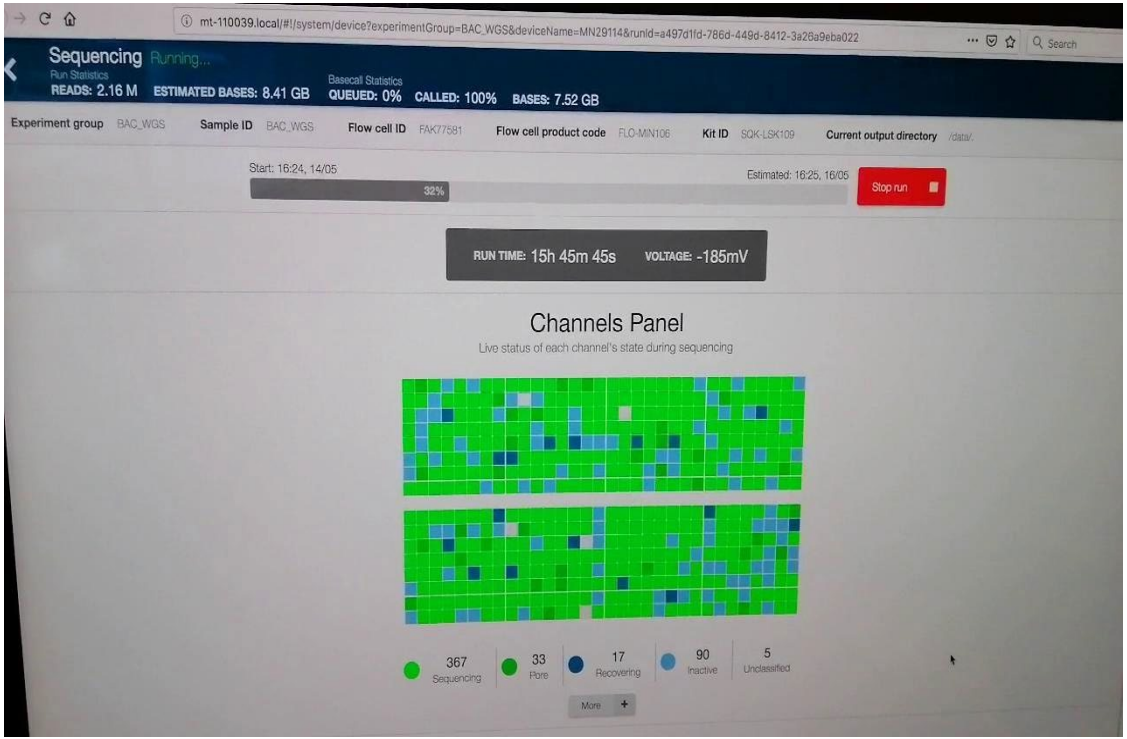


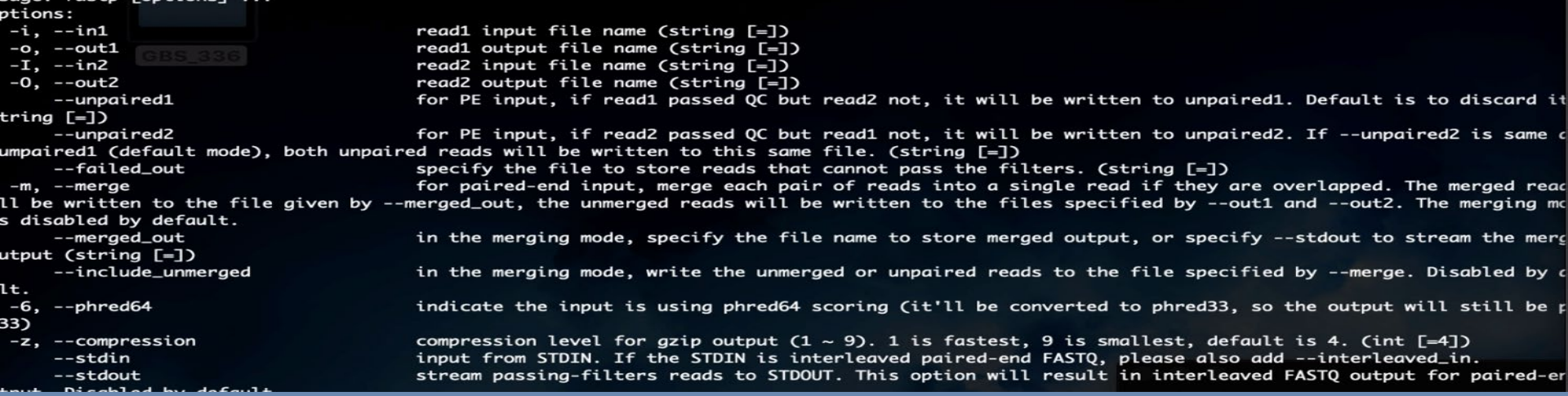






Sequencing run





Bioinformatics: Simplifying Big Data

Dr Gan Han Ming

GeneSEQ

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

WILDERLAB

MAHIDOL UNIVERSITY BIOTEC-NSTDA

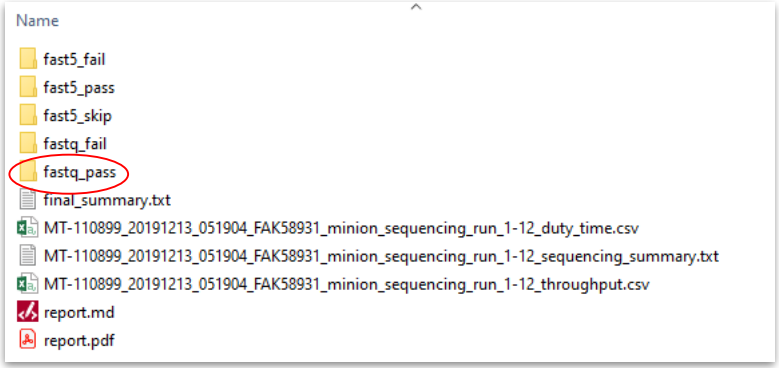
GeneSEQ NEXT GENERATION SEQUENCING

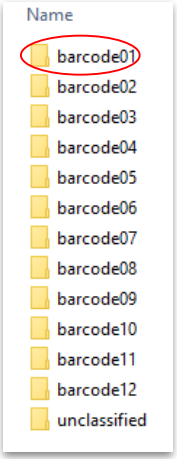
WorldFish

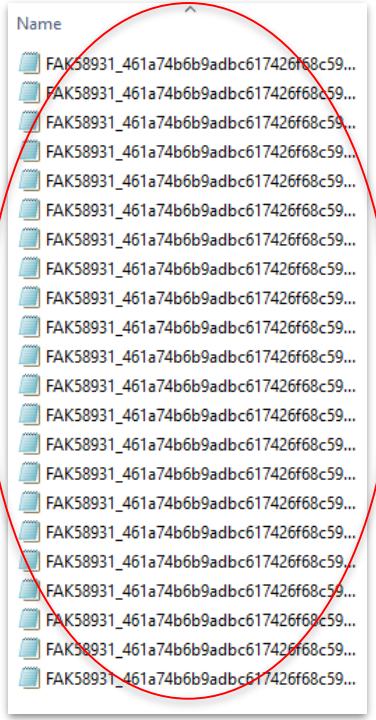
Nanopore Raw Data: Sequencing report

report.pdf

Nanopore Raw Data: File structure







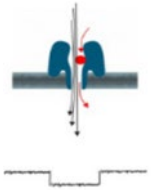
Complexity to Simplicity

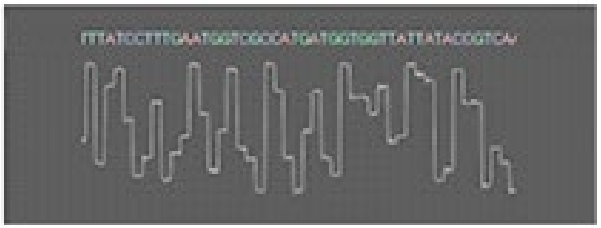
Raw Data

Usable (or “edible”) Information

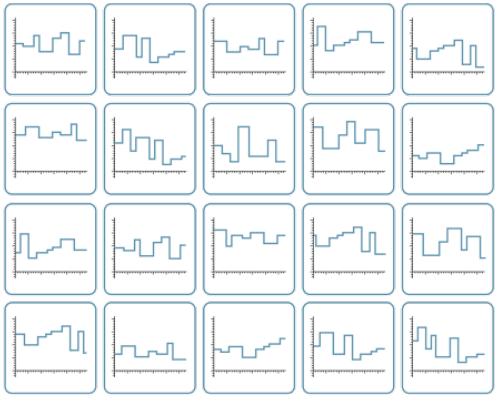
Conversion of Raw Signal Data (fast5) into FastQ file

Biological nanopore





Source: Nanopore



Basecaller - **Machine Learning**
Algorithm to determine DNA sequence from raw data signal

Different Basecaller = Different Sequence Accuracy

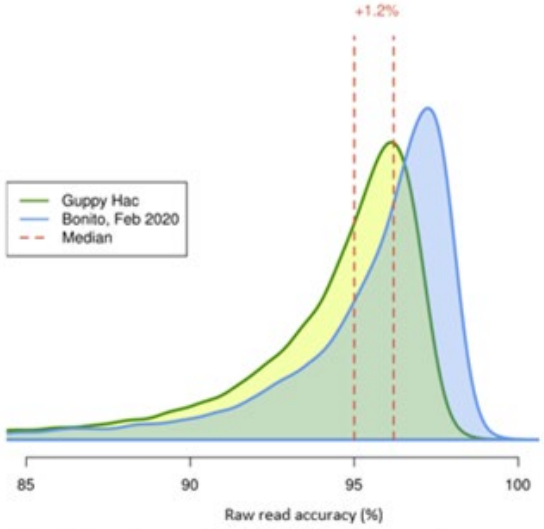


Figure 1: Raw read accuracy of Bonito basecaller on the human reference genome NA12878 against high-accuracy Guppy, currently integrated into MinKNOW onboard nanopore devices.

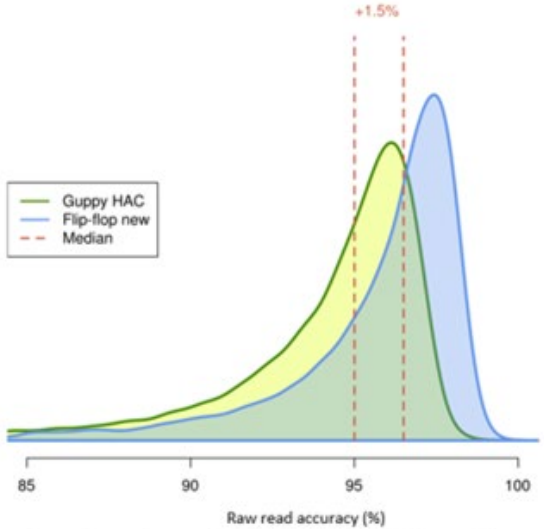


Figure 2: Raw read accuracy of new flip-flop basecaller on the human reference genome NA12878 against high-accuracy Guppy, currently integrated into MinKNOW onboard nanopore devices.

https://nanoporetech.com/about-us/news/new-research-algorithms-yield-accuracy-gains-nanopore-sequencing

Same Raw Fast5 file > Different Basecaller > Different Accuracy
Keep your fast5 file

Illumina (Short Read) FastQ vs Nanopore (Long Read) FastQ

Illumina

```
@MN00696:48:000H2LYGT:1:13104:6285:13781 1:N:0:TAGAGAGA+TACTTCGA
GGTGGTGGTGGAGCTGAGTTCCCCACTTTCTAGTGGACTAGCTCACGCTGGCGGATCCGTTGATCTCGCCATCTTTTCCCTCCACCTCGCGGGGGCCTCCTCTATTCTGGCCACAATAAAATTTATCACCACAATTATTAACATGCGGACT
+
FFFFFFFFF/=AF/F//F=F==/FFFA/F/AFFFFFFF/FFFFFFFFFFFAFAFFFFFFF/FFFFFFFFFFFFF6F/FFFFFFFFFFFFFFFFFFFFFF=FFAFAFFFAFFFFFAFF/F/FFFFFFF=FFFFF=FFAF=FFFAFFFFF6F/
```

Nanopore

```
@1f817505-5d10-49f1-9126-84a5c4777a99 runid=d67dbf8e77ff164429ebf555a6cf6c00078aacb9 sampleid=AmpliconDec2020 read=13682 ch=13 start_time=2020-12-04T08:09:36Z barcode=barcode01
TTATGATTCAGTTACGTATTGCTGTGCTGAAGAAAGTTGTCAGGAGTGTCTTTGTGTTAACCTACTTGCTGAAAATACTAATATTACTACCCGGTTACCTTGTTACGACTTCACCCCAATCATCGCTCCACCTTAGGCGGCTGGCTCCAAAAAGGTTACCCCACCGACTTTCGGGTGTTACAAACACCGATGAAGTCGAGGGTGAGCGAAG
GATATTTAAAGACCGAAGACGATGATGTCGCCGTGAACATTATCGGTCAGGACCGTTTTTCAATTCCAGCTTCATGTAGGCGGTGCAGCCTGTCCGAACGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTACGCTCTTCCCGCCGTCCCATTGCTAGCGTGGTAGCCCGGTCATAAGGGCATGTTGATTTGACGTCATCCCCA
CCTTCCTCGGTTTGTCACCGGCAGTCACCTTAAAGTGCCCAACAATGAGCGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCGACGACAACCATGCACCACACCTATCGCTCTGCTCCCGAAGGAAAGCCCTATCTCCGGGACTTTCAGGATATCAAGACCTGTTGAGTTCTTCGCGTTGC
TTCCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCGGCAGGTTCCAGCCTTGCAGCCGTACTCCCCAGGCGAATTCTTAATGCGTTAACTTCAGCACTAAGGGCGGCCTCTTCGCACACTCATCGTTTACGGCGTGGACTTCTGGGAGGTATCTAGTCTGTTTGCTCCCCACGCTTTCGCACCTCAATGTCAGTTAC
AGACCGAAGTCGCCACTGGTGTTCCTCCATACTCTCTGCATTTCACCGCTACACATGGGCCCTTTCCTCTTTCTGCACTCAAGTCTCCCGGGCACCAATGACCCTCCGTTGAACCGTGAGGCTTTCTATCAGGCAAGTCACCTGCGCGCGCTGCGCCCAATAATTCCGGTTAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCGTAG
TTGGTGGCTCTGGTTAGGTACCGTCAAATTGCCAGCATTCAACTAGCACTTGTTCTTCCCCTGTTAACAGGGTTTACGACCCAGCCTTCATCGCTCACGCGGCGTTGCTCCGTCGGGCTTTCGTCCGTACGGAAGGTACCCTACTGCTGCCTCCGTAGGAGTCTAGGCCGTGTCTCAGTCCCAGTGTGGCCGATGCCTCTCAGGTCGGCTA
CGCATCTCGTTACCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGGGTCCATCCATAAGTGACAGCCAAGCCTTATTTCAATTCGAACCATACAGTTCAAAATGTAATGTCGGTATTAGCCCCGGTTTCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCATTAATTTATACTCACCGTCCGCCGCTAACTGGCGAGCCAAGC
TCAATCATTCGCCCGACTTGCATGTATTAGGCGCATACGCCAGCGTTCATCACGGGCCAGAATCAAACTCT
+
%&$%%$&/4/855).14'(,25=;>9;:786*3724=?8:7&-,&030),:=@8@ACC?<<51+.--+--()((''%%$)$$$&)&&&&$%%$&8(%/003+:84477655;)98774660,2'&$$',05547;8=>55>><6:::0*+('$-/52,8993)018679A:<(+5:3>;7;55)'$&&$#'%%#&)$%$&&(#&%#%%&&
)&%$%%$%%$$&$&$$$$$%%%#'$'%#'$$$&$$'%$%$#%$%%$'#*($$$%#%$$#$,&.715>4C?BCCFA@1996--**0/463'%(((/('',&.+1:2:>BCB=?=;9;?<14<@;725*,)*'(-3.**$$%+.466))&%035<;:9881&#%$#&))('$&%&&%)4/*,+*'+'%%%$$&&+47665794>AD>=64
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<6%%*'')&/<(,51*0)'&56*41&)&&&63,'''0115()1638::;=><AA)<7/00(%#&(()&#&&.030..666<($&=><;200,(004&(-2-175670./$*/23)&&((-766=:4%.(40753)$'%**()+.-0//)))0&(4626,-98/'(7:=><@=?=@+$%767946;9<64)+0.3'%'/07634;C?9:>;=
B?C=A>;632-'0,/445;78*2369:32,;A<;<%'@;5=EAC>?5763)(*;=<=EG>;;8=;9864,'$#%'*&+($$$%(+$,(1*''+$)*,%)(47?4/2<:;84.%)&/.%'((''*-1=>886:>A==6%%#08;<<::-++.&%+,013--+0588781((+$%&10''$''+,./9=;9,,+(+/*&$)$$$('$$&%($%
&)&(&(*+/*$$%$'+*..&#./+&&)'$$%%$$$$#$%%$')+')+(,/.&'$%$"%)*1346/0+./2:
```
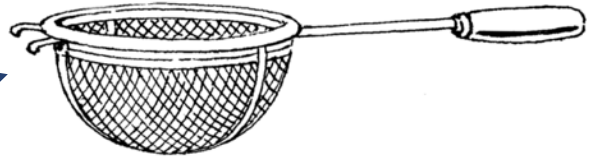
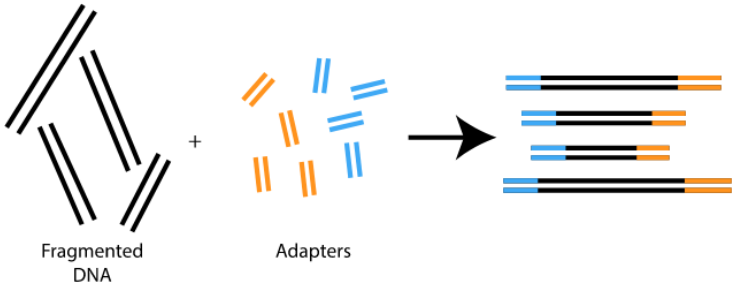

Filtering and trimming reads

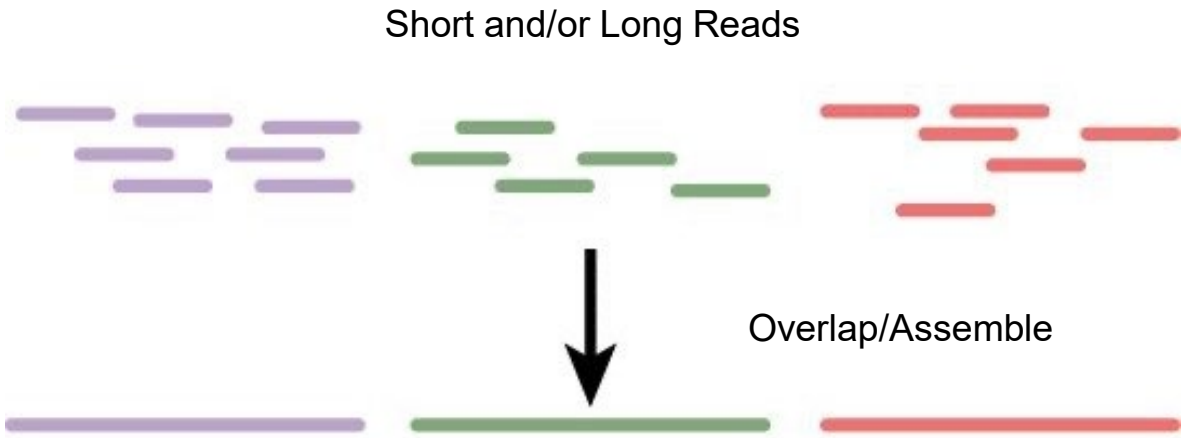
- Adapter read through
- Low-quality reads (low accuracy)
- Filter = to remove completely
- Trimming = to remove certain part of the DNA reads





https://snipcademy.com/ngs-techniques

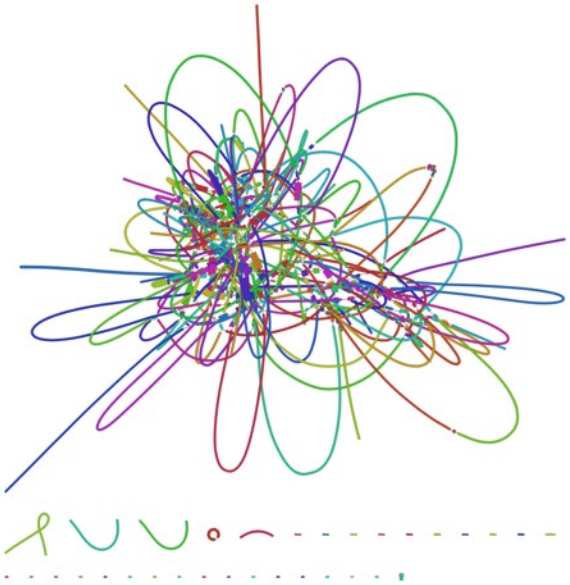
Piecing the “clean” puzzles together

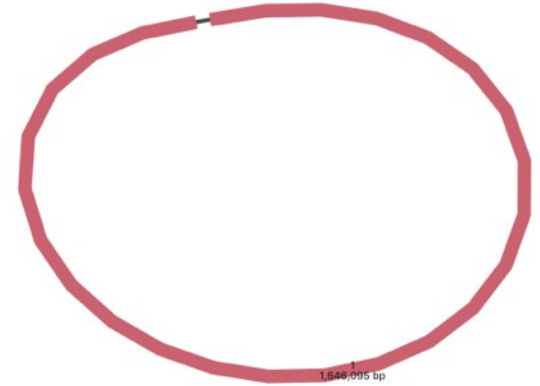


Contigs - overlapping sequence data

Genome assembly statistics outcome depends on read length

Short Reads Assembly Graph:
Multiple Contigs and unresolved knots





Long Reads - Resolve repeats - Simpler Graph

Major currencies in NGS

	Approximate disk storage size
Fast5 Raw basecall signal (but specific to Nanopore) Large but can be re-basecalled to give improved quality	800 Mb
FastQ Raw data (can be submitted to NCBI SRA)	100 Mb (8x smaller than fast5)
Fasta Assembly file (draft or complete) usually similar size as the genome)	2.1 Mb (A typical GBS genome)

Key Metrics of Genome Assembly (Fasta file)

Number of contigs

N50

Total Assembled Length

GC content

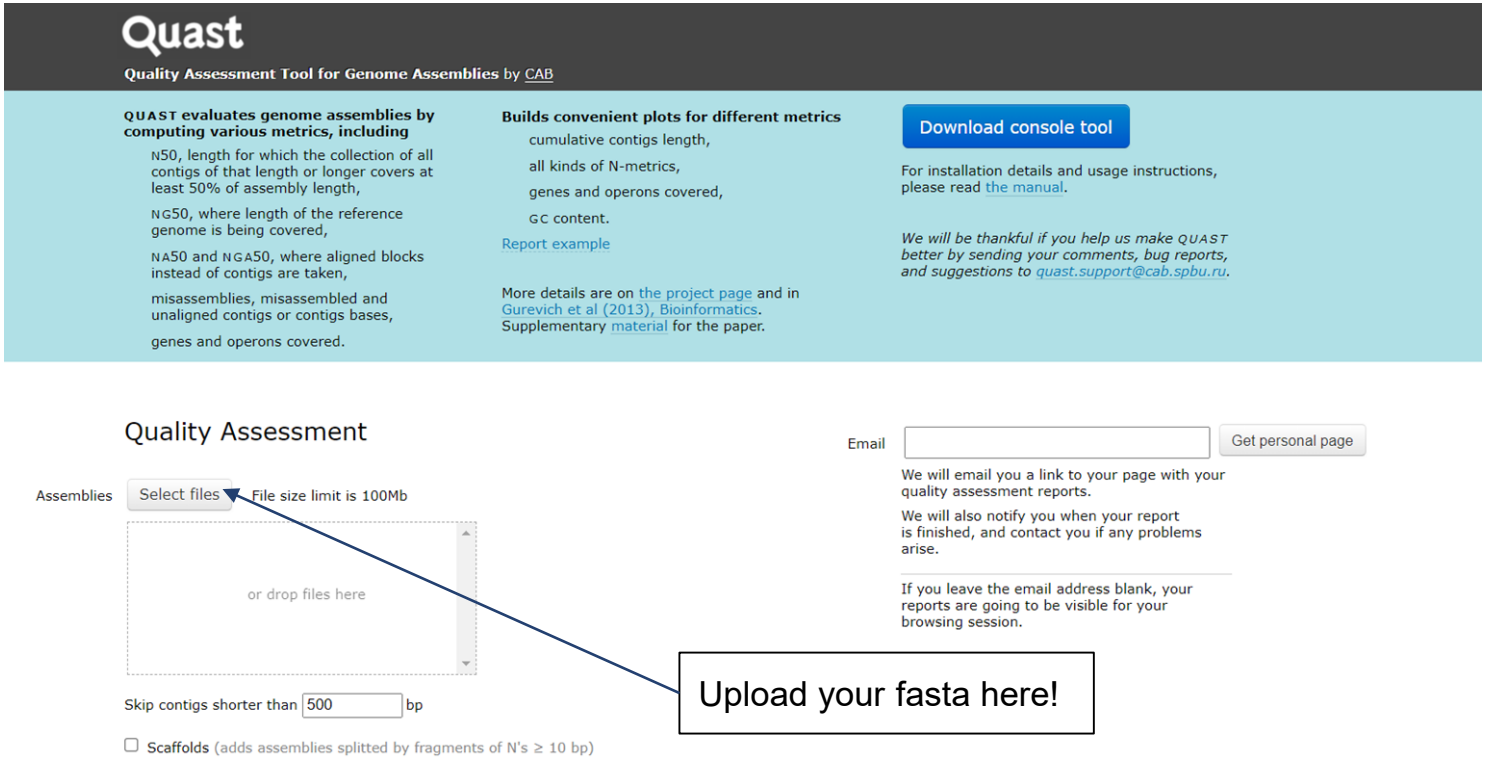
Key Metrics of Genome Assembly

- Number of contigs - Ideal Assembly = 1 contig --- 1 chromosome
- N50 = Shortest contig length at 50% of total assembled length
- Total Assembled Length = Should be similar to expected size
- GC content = No major deviation from other closely related strains

Web-based tools

- Upload **Fasta (or FastQ)** files
- Open-source = Free to use
- Peer-reviewed
- Doesn’t rely on your hardware
- Internet Connection (cloud)

Quality Assessment of Genome Assemblies
QUAST web server



Source: http://cab.cc.spbu.ru/quast/

QUAST Output

Quast

Quality Assessment Tool for Genome Assemblies by CAB

NF_3

01 August 2021, Sunday, 04:19:03

View in Icarus contig browser

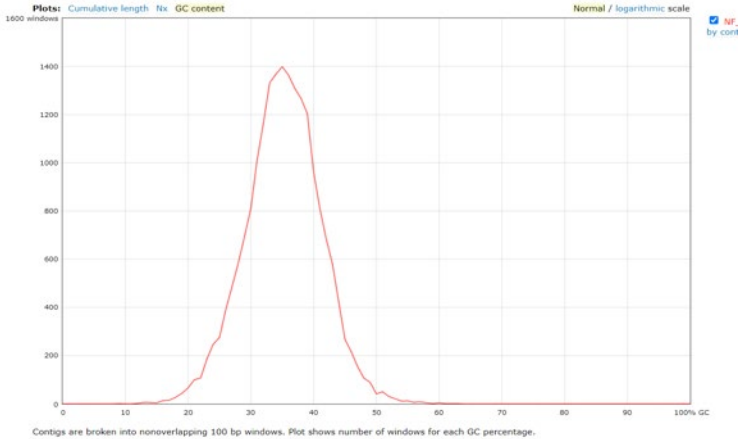
Download report

Text, TSV and Latex versions of the table, plots in PDF.

Additionally, detailed contigs and genome statistics.

All statistics are based on contigs of size >= 500 bp, unless otherwise noted (e.g., "# contigs (>= 0 bp)" and "Total length (>= 0 bp)" include all contigs).

Statistics without reference	NF_3.contigs
# contigs	19
# contigs (>= 0 bp)	19
# contigs (>= 1000 bp)	17
# contigs (>= 5000 bp)	16
# contigs (>= 10000 bp)	15
# contigs (>= 25000 bp)	13
# contigs (>= 50000 bp)	10
Largest contig	491 086
Total length	1 996 902
Total length (>= 0 bp)	1 996 902
Total length (>= 1000 bp)	1 995 577
Total length (>= 5000 bp)	1 990 605
Total length (>= 10000 bp)	1 981 044
Total length (>= 25000 bp)	1 936 495
Total length (>= 50000 bp)	1 830 621
N50	261 619
N75	140 661
L50	3
L75	5
GC (%)	35.18
Mismatches	
# N's	0
# N's per 100 kbp	0



Source: http://cab.cc.spbu.ru/quast/

Assessment of Genome Completeness
BUSCO 5

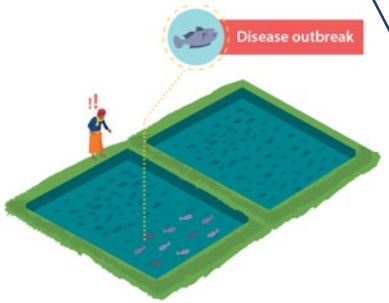
Assembled Genome -> Identification of Conserved Gene (Bacteria ODB10) -> Summarize

100% complete and single-copy gene = target
High Duplicated rate and Low Single-copy rate (S:0%,D:100%) = possible contamination
High fragmented rate indicates fragmented assembly

```
# BUSCO version is: 5.0.0
# The lineage dataset is: bacteria_odb10 (Creation date: 2020-03-06, number of species: 4085, number of BUSCOs: 124)
# Summarized benchmarking in BUSCO notation for file /media/gs/3TB/Jerome_WGS/Final/keep/Presentation_Rename/USM_3.contigs.fasta
# BUSCO was run in mode: genome
# Gene predictor used: prodigal

        ***** Results: *****

        C:99.2%[S:99.2%,D:0.0%],F:0.8%,M:0.0%,n:124
        123     Complete BUSCOs (C)
        123     Complete and single-copy BUSCOs (S)
        0       Complete and duplicated BUSCOs (D)
        1       Fragmented BUSCOs (F)
        0       Missing BUSCOs (M)
        124     Total BUSCO groups searched
```

Genome-based diagnosis of pathogens in aquaculture

NGS
(MinION/Illumina)

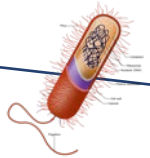


Pathogen Species ID and epidemiology (MLST)

Virulence factor identification

Antibiotic susceptibility

Molecular serotyping
(vaccine formulation)



Management of disease outbreak at farm level

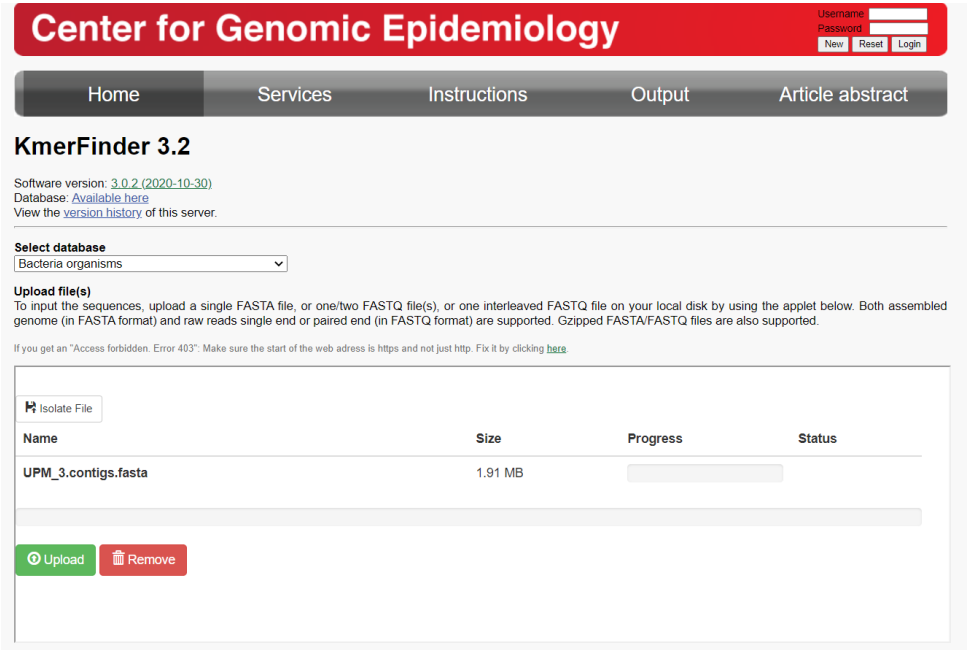
Web-based Species ID, MLST, Serotype, AMR identification

Center for Genomic Epidemiology (CGE)

Source: http://www.genomicepidemiology.org/services/

CGE: Taxonomic Classification (A sanity check)

A Fast K-mer based approach (Kmer finder)



- User-Friendly
- Select Database
- Select Your Genome fasta/fastq file (isolate)
- Upload
- Species ID

Source: https://cge.cbs.dtu.dk/services/KmerFinder/

CGE: Taxonomic Classification (A sanity check)

Center for Genomic Epidemiology

Home Services Instructions Output

KmerFinder-3.2 Server - Results

NF_1

KmerFinder 3.2 results:

Template	Num	Score	Expected	Template_length	Query_Coverage	Template_Coverage	Depth	tot_query_Coverage	tot_template_Coverage	tot_depth	q_value	p_value
NZ_UHIL01000001.1 Vibrio parahaemolyticus strain NCTC10903, whole genome shotgun sequence	200463	84406	8	110506	47.29	78.01	0.76	47.29	78.01	0.76	84379.93	1.0e-26
NZ_CP014047.2 Vibrio parahaemolyticus strain ATCC 17802 chromosome 2, complete sequence	409450	46084	8	64881	25.82	71.11	0.71	25.97	71.41	0.71	46059.81	1.0e-26
NC_013456.1 Vibrio antiquarius chromosome 1, complete sequence	85991	1503	19	110687	0.84	1.37	0.01	9.16	14.81	0.15	1446.53	1.0e-26

Center for Genomic Epidemiology

Home Services Instructions Output

KmerFinder-3.2 Server - Results

USM_3

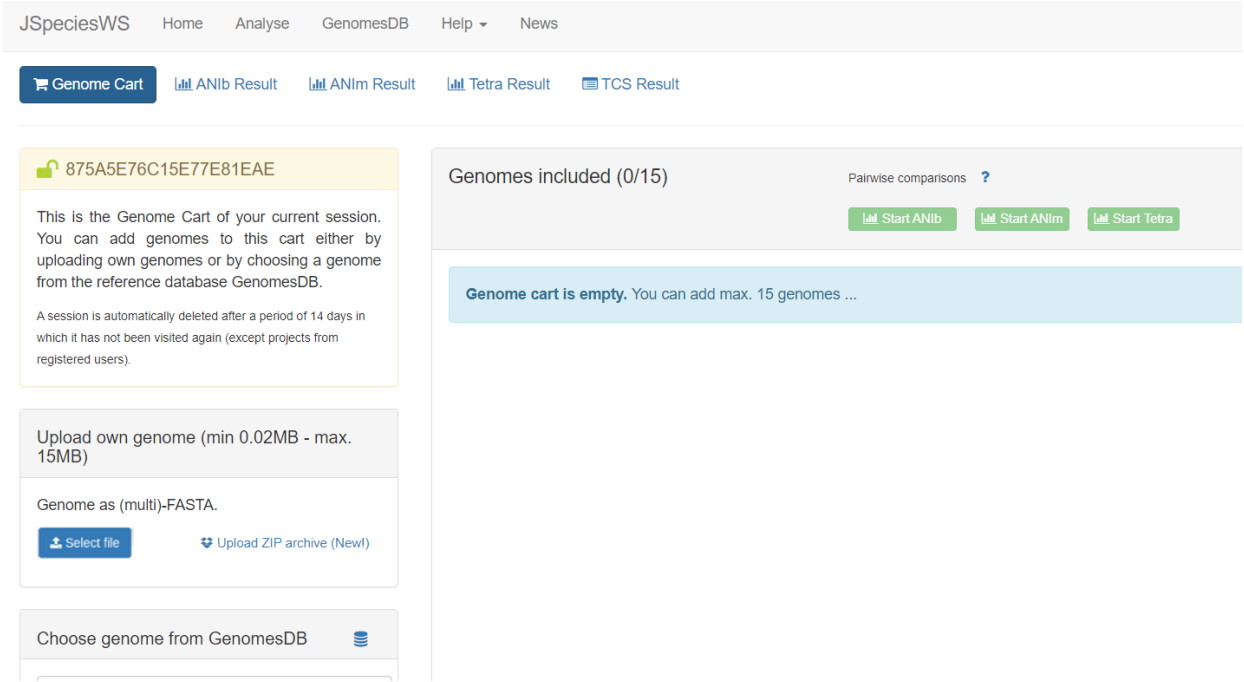
KmerFinder 3.2 results:

Template	Num	Score	Expected	Template_length	Query_Coverage	Template_Coverage	Depth	tot_query_Coverage	tot_template_Coverage	tot_depth
NZ_JMIB01000004.1 Photobacterium galatheae strain S2753 contig0005, whole genome shotgun sequence	315626	1860	1	12265	1.03	15.19	0.15	1.03	15.19	0.15
NZ_JMIB01000006.1 Photobacterium galatheae strain S2753 contig0007, whole genome shotgun sequence	315628	1851	1	9514	1.02	19.35	0.19	1.02	19.35	0.19

Average Nucleotide Identity Calculation

JSpecies Webserver

In-silico genome-genome hybridization



Source: http://jspecies.ribohost.com/jspeciesws/

Average Nucleotide Identity Calculation
JSpecies Webserver

Compare with TYPE STRAIN (**Descendants of the original isolates used in species and subspecies descriptions**). Not all strains are type strains!

ANIm Result Matrix | ANIm Result by Genome

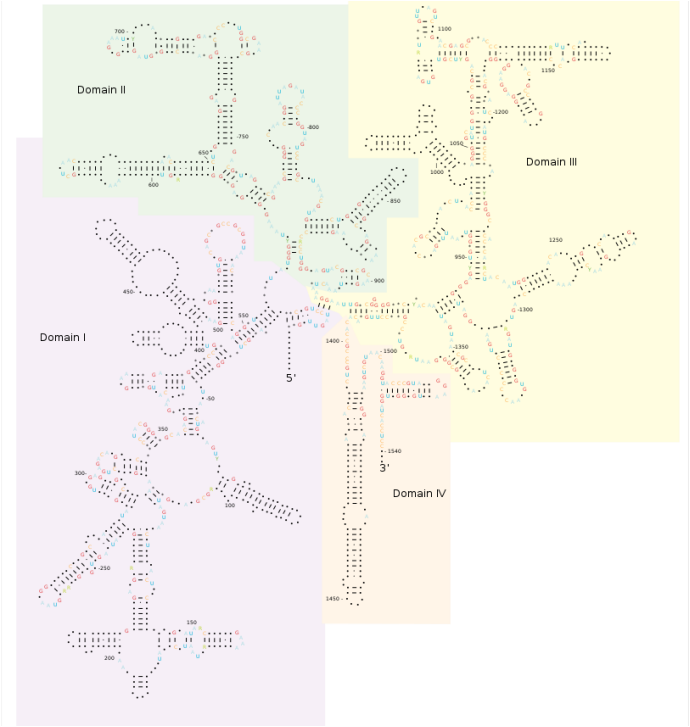
Show ANIm and [aligned nucleotides] [%] ▾ | Download as .csv

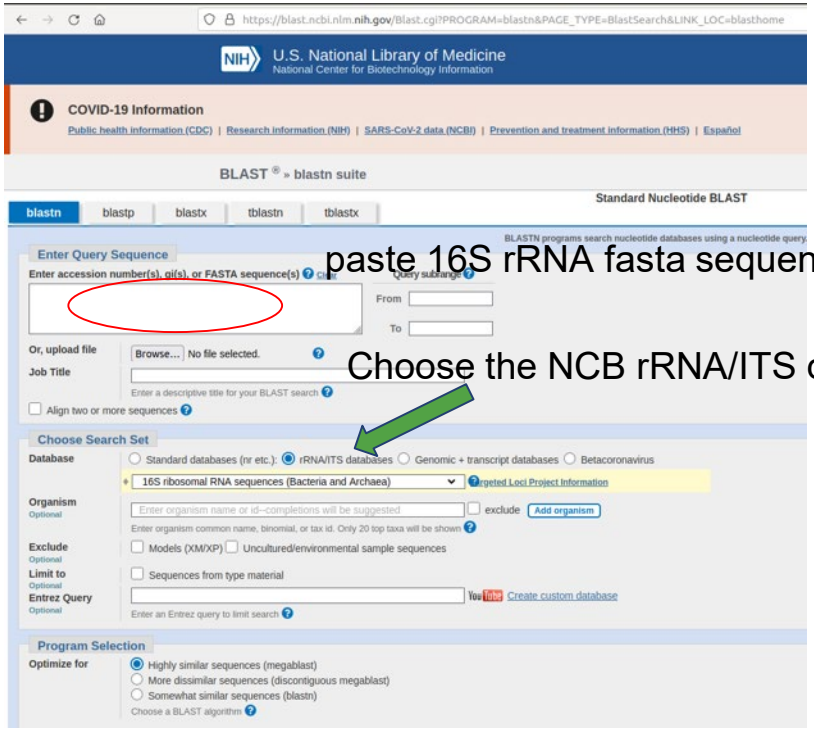
Legend: Above cutoff (> 95%) | Below cutoff (< 95%) | Suspicious alignment!

	USM 3.contigs.fasta	Vibrio parahaemolyticus ATCC 17802 [T]	Vibrio alginolyticus FDAARGOS 97 [T]	Vibrio antiquarius EX25 [T]	Vibrio diabolicus CNCM I-1629 [T]	Vibrio harveyi FDAARGOS 109 [T]	NF 1.contigs.fasta
USM 3.contigs.fasta	*	83.98 *(1.39)*	84.54 *(1.37)*	85.17 *(0.92)*	85.12 *(1.22)*	83.98 *(1.53)*	84.45 *(1.41)*
Vibrio parahaemolyticus ATCC 17802 [T]	84.03 *(1.48)*	*	86.79 *(58.71)*	87.57 *(59.01)*	87.39 *(63.21)*	85.30 *(46.04)*	**98.58** *(93.29)*
Vibrio alginolyticus FDAARGOS 97 [T]	84.57 *(2.28)*	86.78 *(57.65)*	*	92.14 *(81.04)*	92.19 *(86.04)*	86.38 *(44.02)*	86.81 *(59.47)*
Vibrio antiquarius EX25 [T]	84.97 *(1.67)*	87.56 *(61.52)*	92.14 *(85.28)*	*	**98.11** *(92.71)*	85.56 *(41.26)*	87.61 *(62.70)*
Vibrio diabolicus CNCM I-1629 [T]	84.97 *(1.32)*	87.38 *(62.35)*	92.19 *(85.15)*	**98.11** *(87.07)*	*	85.53 *(41.87)*	87.41 *(62.80)*
Vibrio harveyi FDAARGOS 109 [T]	84.01 *(2.25)*	85.31 *(41.29)*	86.39 *(40.15)*	85.57 *(35.78)*	85.54 *(38.82)*	*	85.35 *(41.85)*
NF 1.contigs.fasta	84.48 *(1.42)*	**98.58** *(90.68)*	86.81 *(57.53)*	87.61 *(57.81)*	87.41 *(61.88)*	85.35 *(44.39)*	*

Species Identification based on 16S rRNA similarity

NCBI-BLAST Webserver

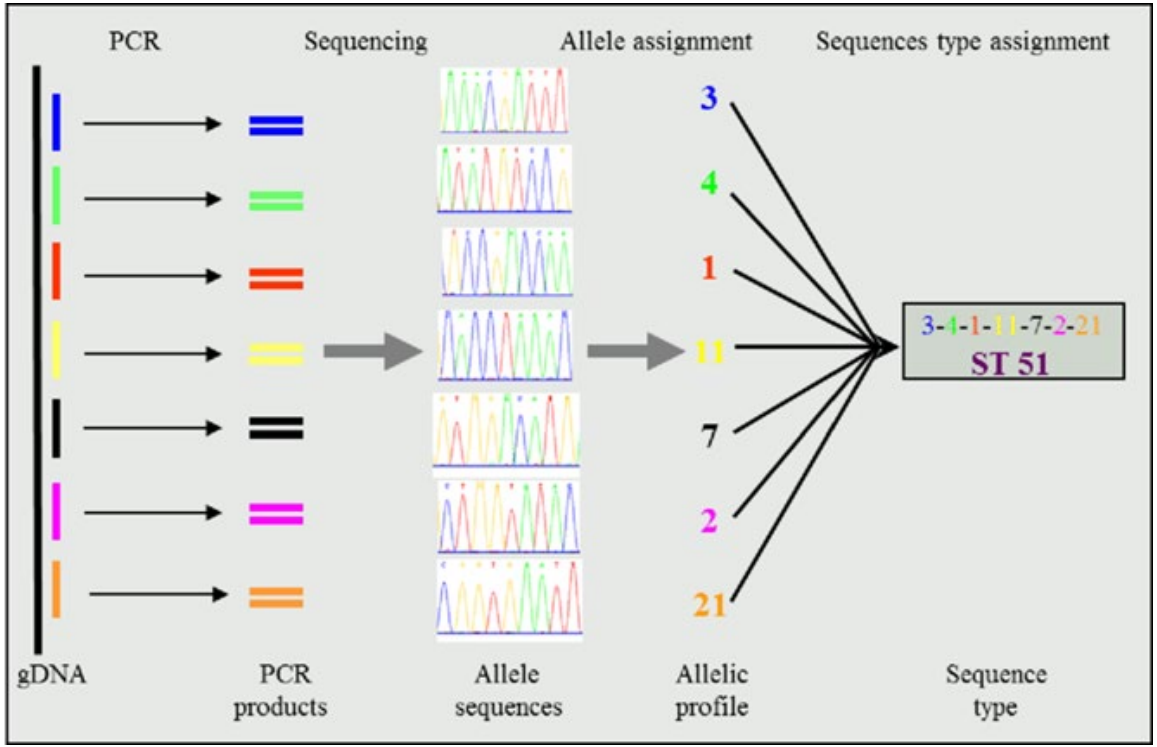




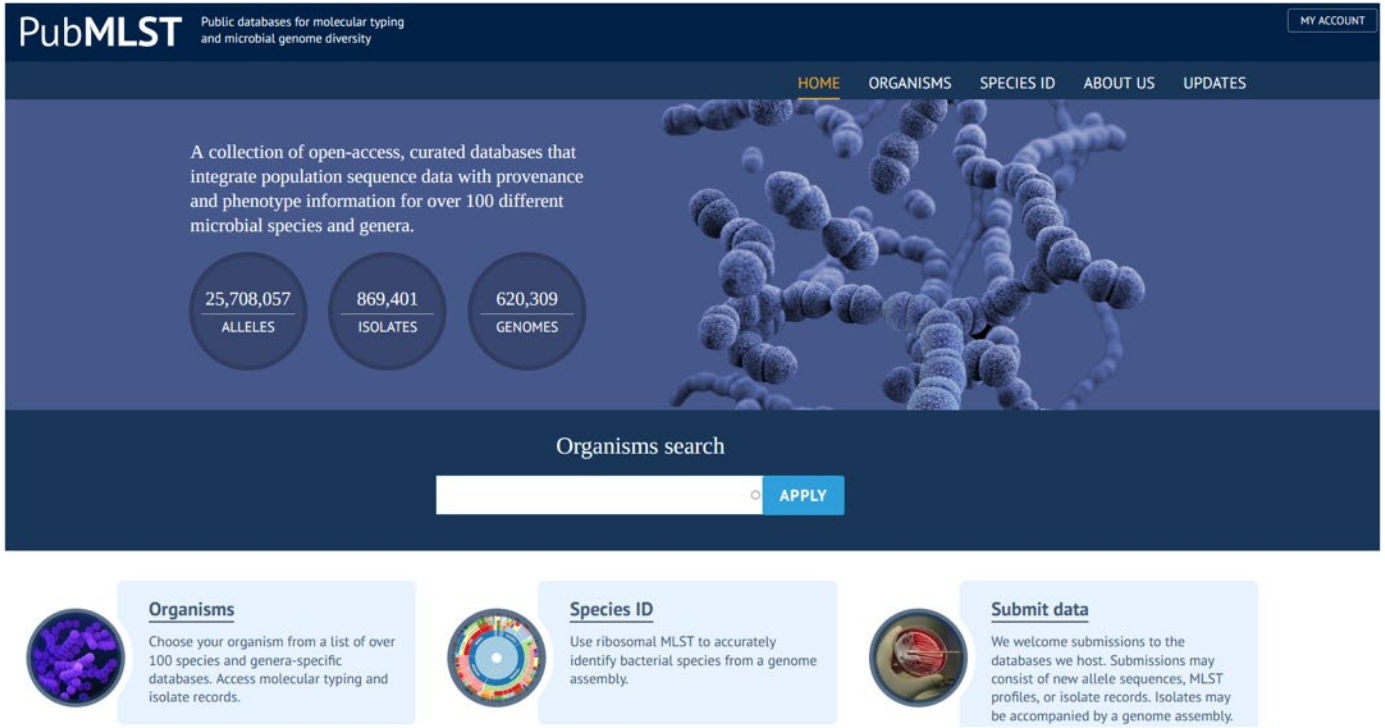
paste 16S rRNA fasta sequence

Choose the NCB rRNA/ITS databases

Multi Locus Sequence Typing (MLST)

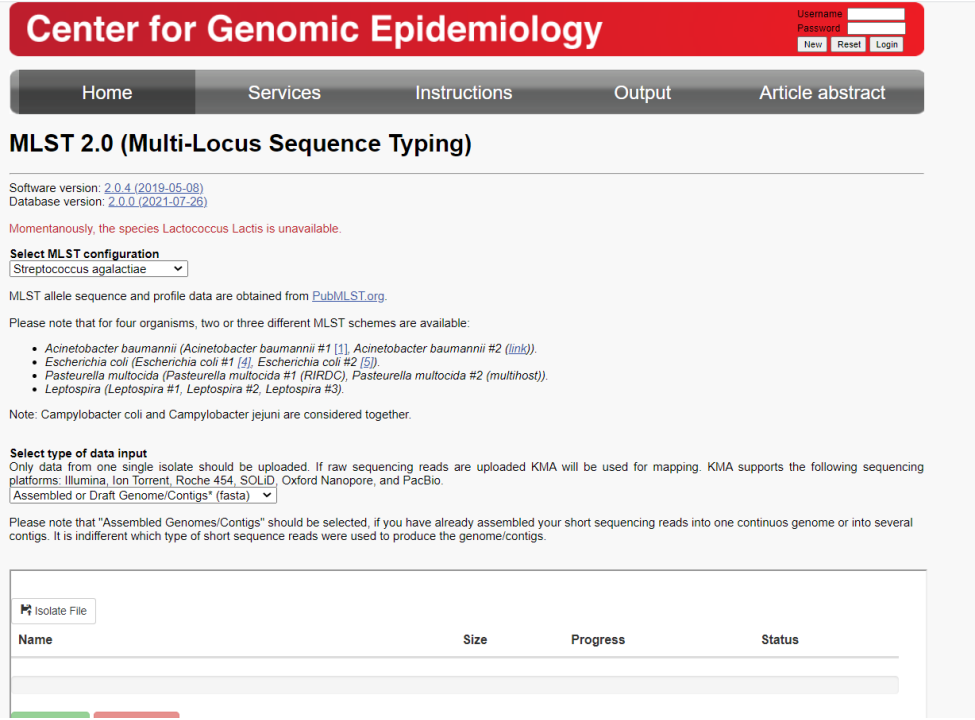


Public databases for molecular typing and microbial genome diversity



Source: https://pubmlst.org/

CGE: Genome-Based MLST
MLST 2.0 (Multi-Locus Sequence Typing)



Source: https://cge.cbs.dtu.dk/services/MLST/

- Alternative to conventional MLST based on PCR / Sanger
- Fast and Efficient
- Digitalize
- Less Error-prone

CGE: MLST from assembled contigs or raw data

MLST 2.0 (Multi-Locus Sequence Typing)

Center for Genomic Epidemiology

Home | Services | Instructions | Output

MLST-2.0 Server - Results

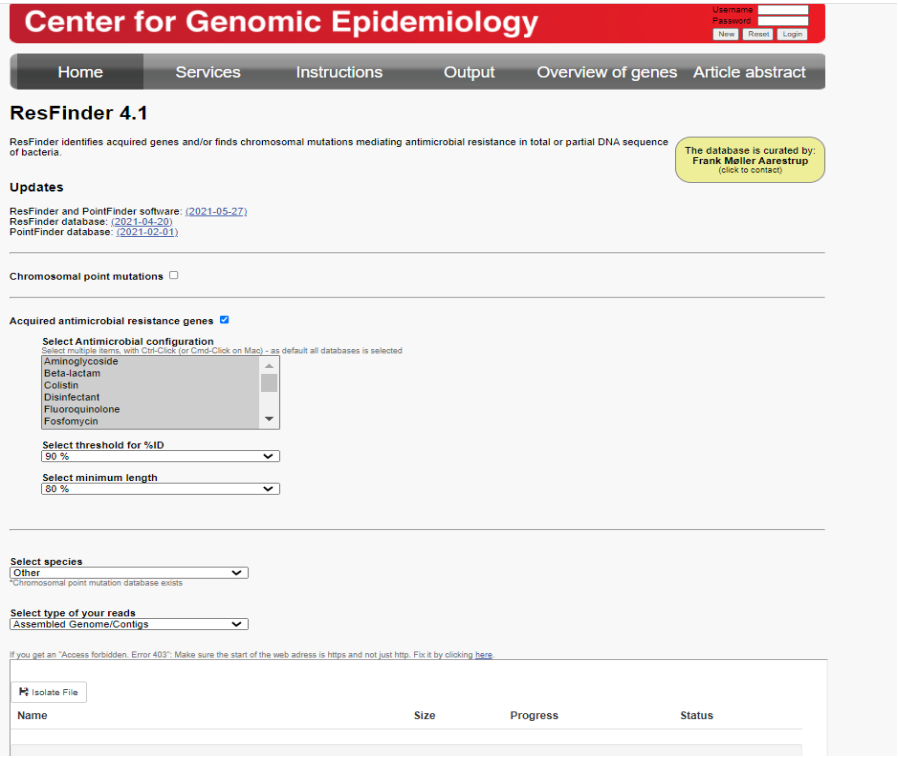
mlst Profile: *sagalactiae*

Organism: *Streptococcus agalactiae*

Sequence Type: *283!*

Locus	Identity	Coverage	Alignment Length	Allele Length	Gaps	Allele
adhP	100	100	498	498	0	adhP_9
atr	100	100	501	501	0	atr_7
glcK	100	100	459	459	0	glcK_3
glnA	100	100	498	498	0	glnA_1
pheS	100	100	501	501	0	pheS_5
sdhA	100	100	519	519	0	sdhA_3
tkt	100	100	480	480	0	tkt_134!
tkt	100	100	480	480	0	tkt_2!

CGE: AMR identification from assembled contigs or raw data
ResFinder 4.1



- Homology-based AMR identification
- % ID vs % Length (Specific vs Sensitive)
- Select genome and Upload

Source: https://cge.cbs.dtu.dk/services/ResFinder/

CGE: AMR identification from assembled contigs or raw data

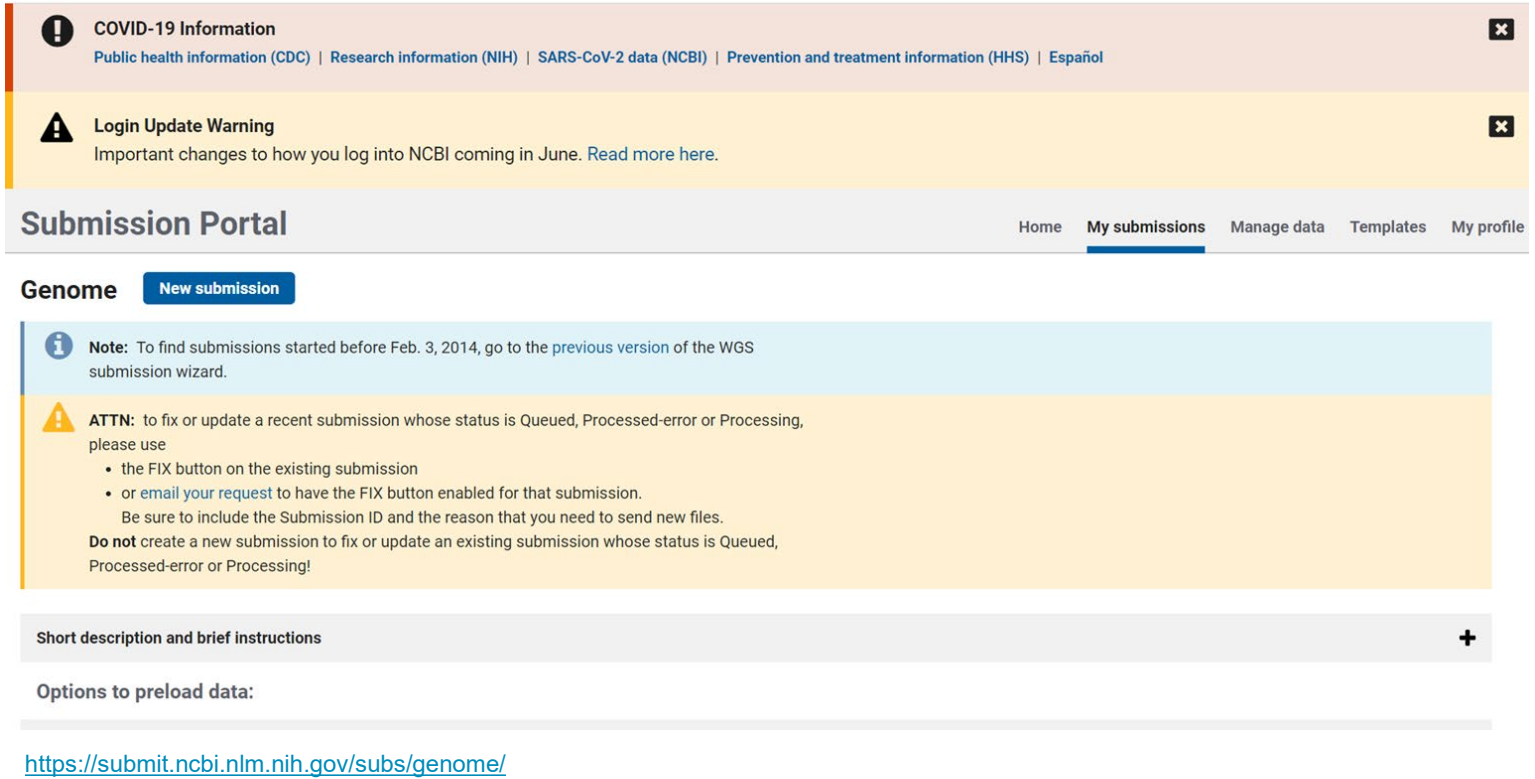
ResFinder 4.1

Macrolide									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
mre(A)	100.0	936/936	1..936	NODE_4_length_152740_cov_230.023915	31574..32509	erythromycin,azithromycin,spiramycin	9420045	U92073	

Tetracycline									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
tet(M)	100.0	1920/1920	1..1920	NODE_3_length_261619_cov_280.568306	233331..235250	doxycycline,tetracycline,minocycline	20546576	AM990992	

Source: https://cge.cbs.dtu.dk/services/ResFinder/

Genome Submission to NCBI



https://submit.ncbi.nlm.nih.gov/subs/genome/

A Typical Annotated Genome by NCBI

- .gbk format with GenBank Accession code for the assembly
- Draft or complete assembly will be accepted
- Unique Accession for each Gene
- Protein Translation
- Start and Stop
- Putative Function description

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                /note="Derived by automated computational analysis using
                gene prediction method: tRNAscan-SE."
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                KLIRYASKSKDVARIFVHPVIKEQLCLQENGKDRSWLRKVRPWWGHHYHFHVRLSCPD
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                PN"
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261 ttatgtgaag ctttaccaaa ccccttt
321 ttagctgcgt tagttgatgc aatgata
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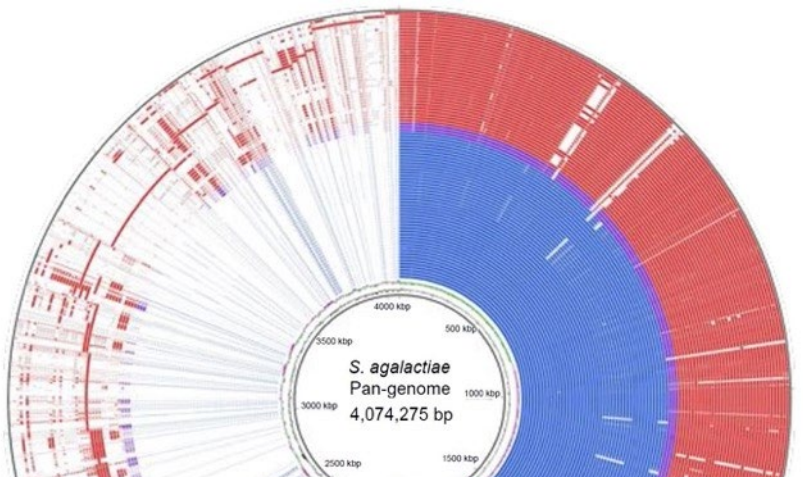


Participant Data Overview

Dr Gan Han Ming

GeneSEQ

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

WILDERLAB

MAHIDOL UNIVERSITY BIOTEC-NSTDA

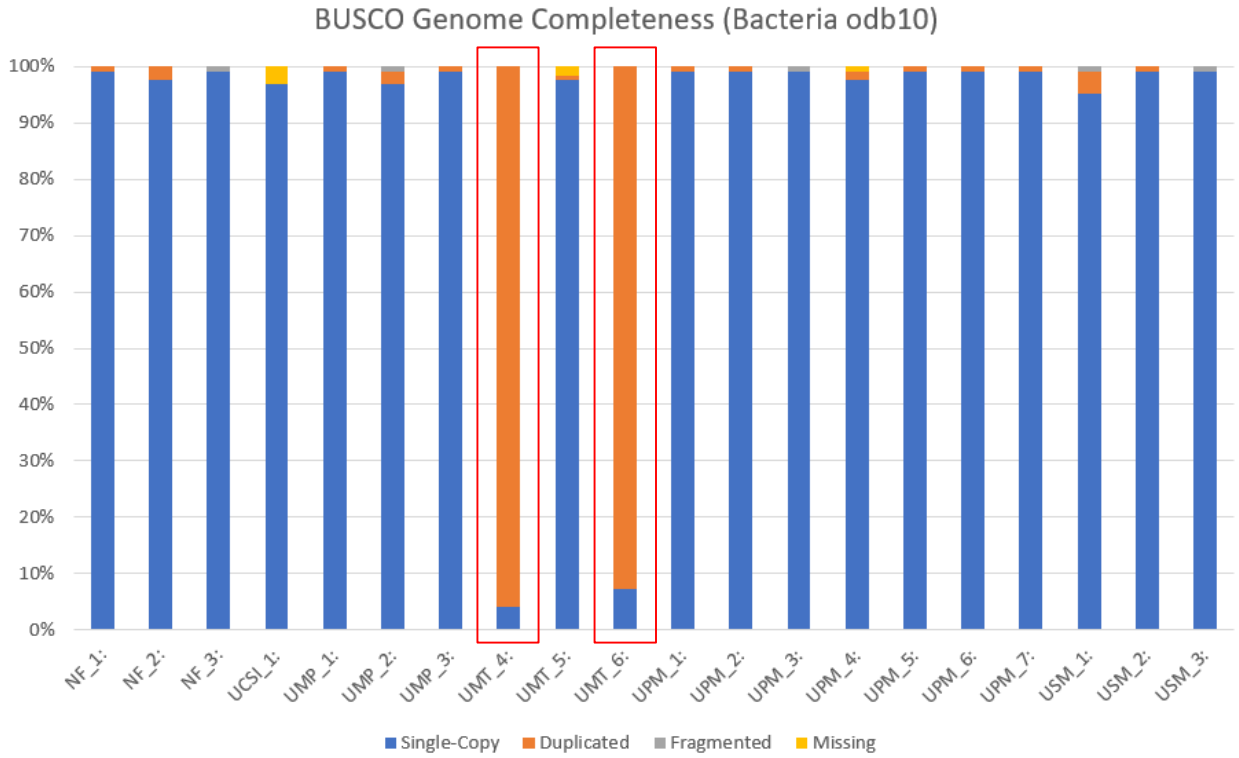
GeneSEQ NEXT GENERATION SEQUENCING

WorldFish

Participants' Analyzed Data
Putative Species Assignment Based on WGS

ID	family	genus	species
UMP_1.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio parahaemolyticus
UMT_6.contigs.fasta	f__Bacillaceae_G	g__Bacillus_A	
USM_2.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio diabolicus
UMP_2.contigs.fasta	f__Bacillaceae_H	g__Priestia	
UPM_7.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio parahaemolyticus
UMP_3.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio parahaemolyticus
UMT_4.contigs.fasta	f__DSM-18226	g__Cytobacillus	
UMT_5.contigs.fasta	f__Enterobacteriaceae	g__Enterobacter	
USM_3.contigs.fasta	f__Vibrionaceae	g__Photobacterium	s__Photobacterium galatheae
UCSI_1.contigs.fasta	f__Enterobacteriaceae	g__Escherichia_C	s__Escherichia_C alba
USM_1.contigs.fasta	f__Bacillaceae_G	g__Bacillus_A	
NF_3.contigs.fasta	f__Streptococcaceae	g__Streptococcus	s__Streptococcus agalactiae
NF_2.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio harveyi
UPM_3.contigs.fasta	f__Streptococcaceae	g__Streptococcus	s__Streptococcus agalactiae
UPM_6.contigs.fasta	f__DSM-18226	g__Cytobacillus	s__Cytobacillus oceanisediminis_B
UPM_4.contigs.fasta	f__Aeromonadaceae	g__Aeromonas	
UPM_2.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio parahaemolyticus
NF_1.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio parahaemolyticus
UPM_5.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio parahaemolyticus
UPM_1.contigs.fasta	f__Vibrionaceae	g__Vibrio	s__Vibrio parahaemolyticus

Assessment of Genome Completeness



MLST on selected strains (*Vibrio parahaemolyticus*)

Sample ID	Species	Sequence Type (ST)	MLST Allele						
NF_1.contigs.fasta	vparahaemolyticus	799	dnaE(28)	gyrB(4)	recA(82)	dtdS(88)	pntA(63)	pyrC(187)	tnaA(1)
UMP_1.contigs.fasta	vparahaemolyticus	-	dnaE(71)	gyrB(13)	recA(97)	dtdS(~461)	pntA(~116)	pyrC(82)	tnaA(26)
UMP_3.contigs.fasta	vparahaemolyticus	-	dnaE(71)	gyrB(13)	recA(97)	dtdS(~461)	pntA(~116)	pyrC(82)	tnaA(26)
UPM_1.contigs.fasta	vparahaemolyticus	1913	dnaE(363)	gyrB(505)	recA(218)	dtdS(442)	pntA(30)	pyrC(303)	tnaA(26)
UPM_2.contigs.fasta	vparahaemolyticus	-	dnaE(~98)	gyrB(264)	recA(~105)	dtdS(~13)	pntA(2)	pyrC(~403)	tnaA(94)
UPM_7.contigs.fasta	vparahaemolyticus	392	dnaE(5)	gyrB(84)	recA(115)	dtdS(74)	pntA(63)	pyrC(159)	tnaA(84)

https://pubmlst.org/bigsdb?page=profileInfo&db=pubmlst_vparahaemolyticus_seqdef&scheme_id=1&profile_id=1913

Profile information for ST-1913 (MLST)

ST	dnaE	gyrB	recA	dtdS	pntA	pyrC	tnaA	clonal complex
1913	363	505	218	442	30	303	26	no

sender: Chrystine Yan, School of Science, Monash University Malaysia and 2. Genomics Facility, Tropical Medicine and Biology Platform, Monash University Malaysia
curator: Narjol Gonzalez-Escalona, Molecular Methods & Subtyping Branch, Division of Microbiology, Office of Regulatory Science, Center for Food Safety and Applied Nutrition, FDA, MD, USA
update history: 1 update show details
date entered: 2018-05-30
datestamp: 2018-05-30

ST-1913 Background

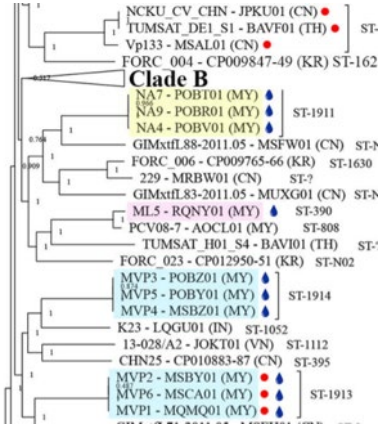
Volume 366, Issue 17
September 2019

< Previous Next >

Genomic characterization of *Vibrio parahaemolyticus* from Pacific white shrimp and rearing water in Malaysia reveals novel sequence types and structural variation in genomic regions containing the *Photorhabdus* insect-related (Pir) toxin-like genes

Chrystine Zou Yi Yan, Christopher M Austin, Qasim Ayub, Sadequr Rahman, Han Ming Gan ✉

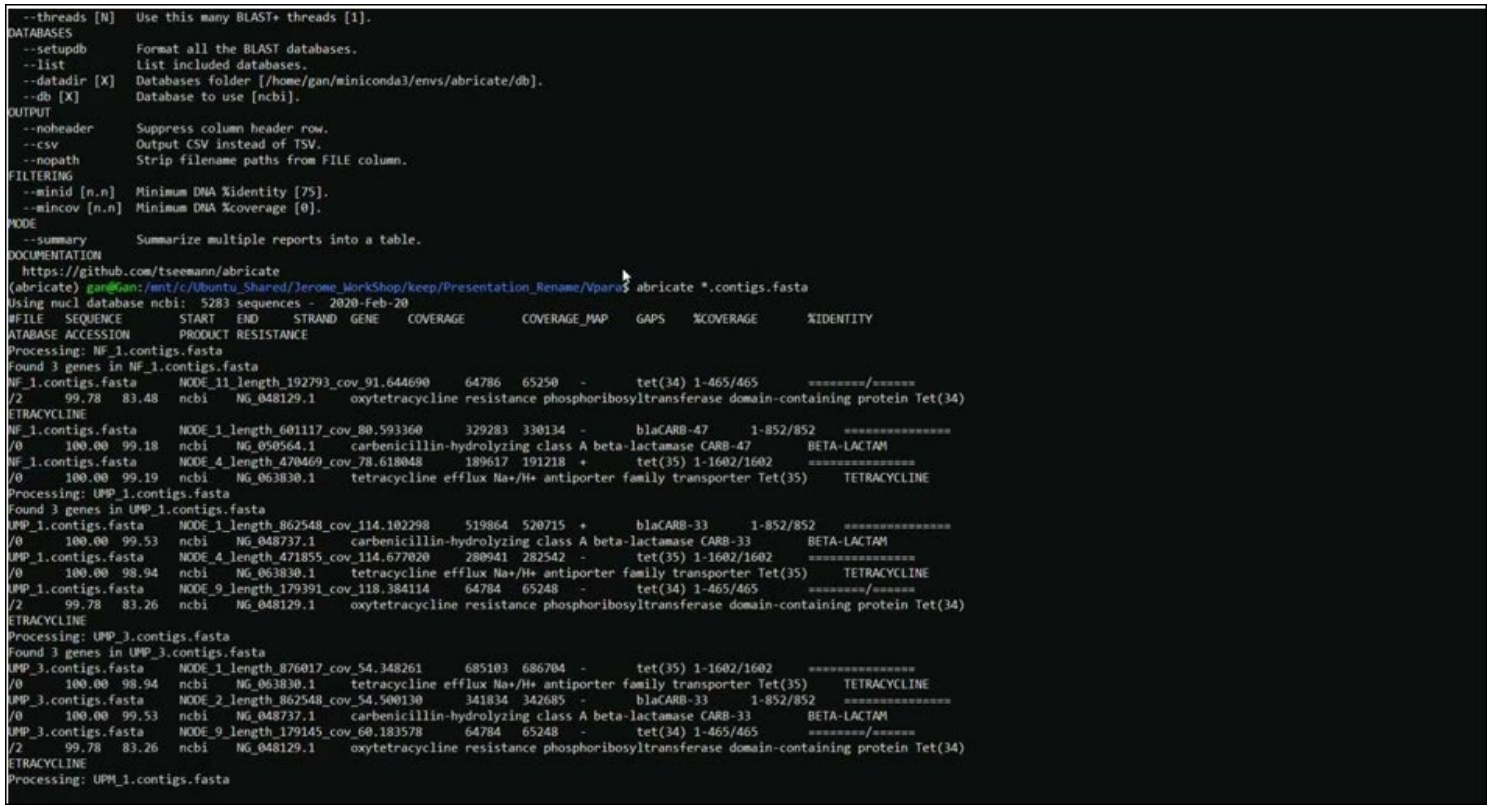
FEMS Microbiology Letters, Volume 366, Issue 17, September 2019, fnz211,
https://doi.org/10.1093/femsle/fnz211
Published: 07 October 2019 **Article history** ▾



Three major structural variants of the pVA plasmid among Asian *V. parahaemolyticus*

Of the 52 sequence types of *V. parahaemolyticus* identified in this current genomic sampling, $pirAB^{Vp}$ could only be detected among isolates from 8 validated (ST-2013, ST-114, ST-150, ST-392, ST-413, ST-970, ST-1166, and ST-1913) and 1 undetermined sequence types (Figure 1).

Linux command line - An example with AMR identification



Visualization of AMR profile among sequenced strains

#FILE	BclI	aph(3'')-Ib	aph(6)-Id	bla1	blaACT-70	blaCARB-26	blaCARB-33	blaCARB-38	blaCARB-42	blaCARB-45	blaCARB-47	blaLHK-6	blaOXA-724	blaP	blaTEM-1	blaVHH-1	catA10	catA2	cepH	dfrA14	dfrA17	fexA	fosB-38141535	fosB_gen	fosD	imiH	lsa(B)	mcr-7.1	mecA1	oqxA9	oqxB9	qnrS2	rphC	sal(A)	satA_Ba	sul1	sul2	tet(34)	tet(35)	tet(45)	tet(A)	tet(K)	tet(M)
NF_1	.	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	99.78	100	.	.	.	.
NF_2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	99.77	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100	100	.	.	.	.
NF_3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100
UCSI_1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
UMP_1	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	99.78	100	.	.	.	.
UMP_2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	99.88	.	.	.	.	.	.	.	.	.	.
UMP_3	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	99.78	100	.	.	.	.
UMT_4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100	.	.	100	.	.	.	100	.	.	.	100	.	.	.	.	.	.	.	100	.
UMT_5	.	100	100	.	100	.	.	.	.	.	.	.	.	.	100	.	.	100	.	100	.	.	.	.	.	.	.	.	.	100	98.95	.	.	.	100	.	.	.	.	.	.
UMT_6	##	.	.	99	.	.	.	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	.	99.76	.	.	.	.	.	.	.	99.35	.	.	.	.	.	.	.	.	.	.
UPM_1	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	99.78	100	.	.	.	.
UPM_2	.	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100	99.78	100	.	.	.	.
UPM_3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100
UPM_4	.	.	.	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	100	.	100	.	.	.	.	100	.	86.48	.	.	.	100	.	.	100	.	.	.	.	100	.	.
UPM_5	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	99.78	100	.	.	.	.
UPM_6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
UPM_7	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	99.78	100	.	.	.	.
USM_1	##	.	.	100	.	.	.	.	.	.	.	.	.	80.84	.	.	.	.	.	.	.	.	31.18	100	.	.	98.99	.	.	.	.	.	99.5	.	98.38	.	.	.	.	100	.	.
USM_2	.	.	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100	99.38	.	.	.	.
USM_3	.	.	.	.	.	.	.	.	.	.	.	11.08	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

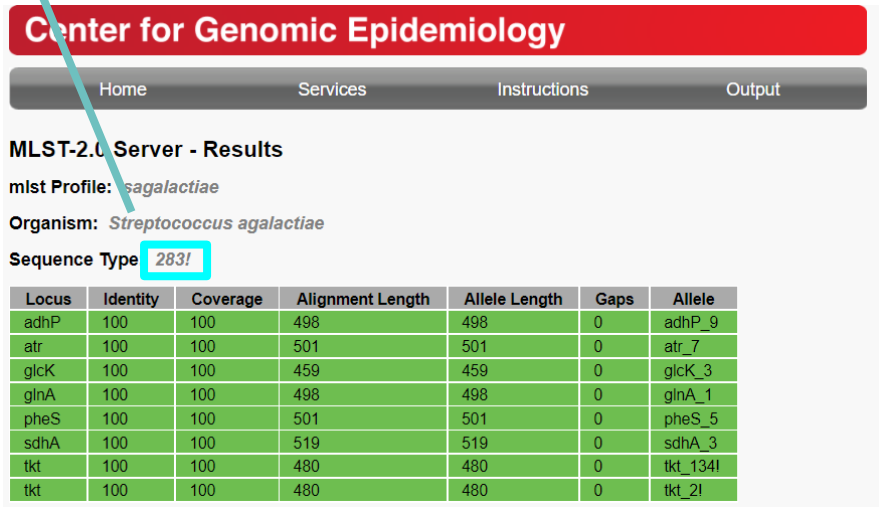
Source: https://github.com/tseemann/abricate

MLST on selected strains (GBS)

(1) Using Linux based approach (https://github.com/tseemann/mlst)

```
NF_3.contigs.fasta      sagalactiae     283     adhP(9) pheS(5) atr(7)  glnA(1) sdhA(3) glcK(3) tkt(2)
UPM_3.contigs.fasta     sagalactiae     283     adhP(9) pheS(5) atr(7)  glnA(1) sdhA(3) glcK(3) tkt(2)
```

SAME RESULTS

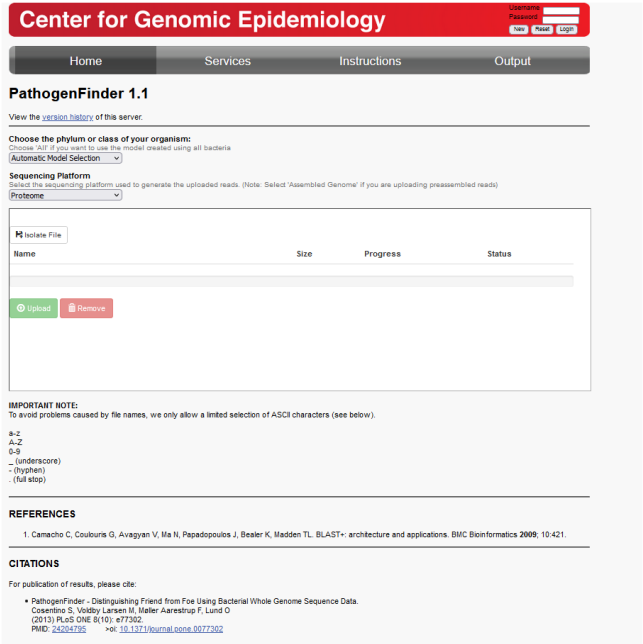


Locus	Identity	Coverage	Alignment Length	Allele Length	Gaps	Allele
adhP	100	100	498	498	0	adhP_9
atr	100	100	501	501	0	atr_7
glcK	100	100	459	459	0	glcK_3
glnA	100	100	498	498	0	glnA_1
pheS	100	100	501	501	0	pheS_5
sdhA	100	100	519	519	0	sdhA_3
tkt	100	100	480	480	0	tkt_134!
tkt	100	100	480	480	0	tkt_2!

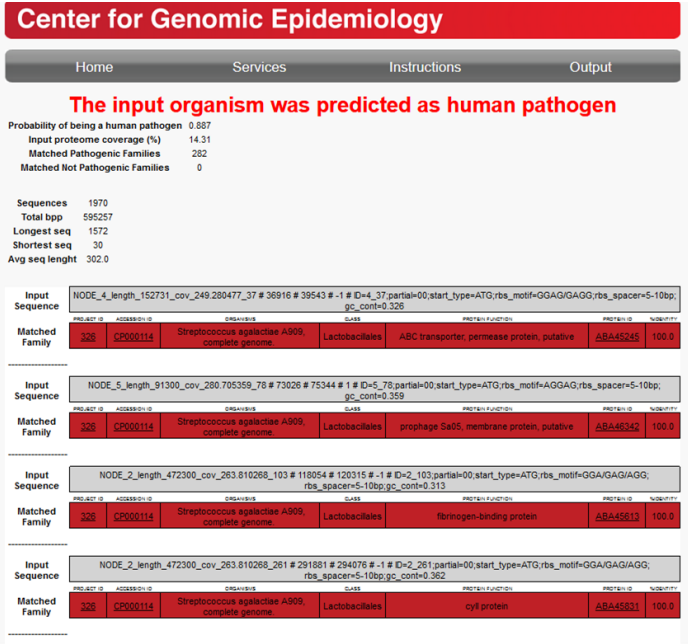
(2) Rapid and precise alignment draft genome/contigs (fasta) against redundant databases with KMA

CGE: ST-283 pathogenicity towards human hosts

PathogenFinder 1.1

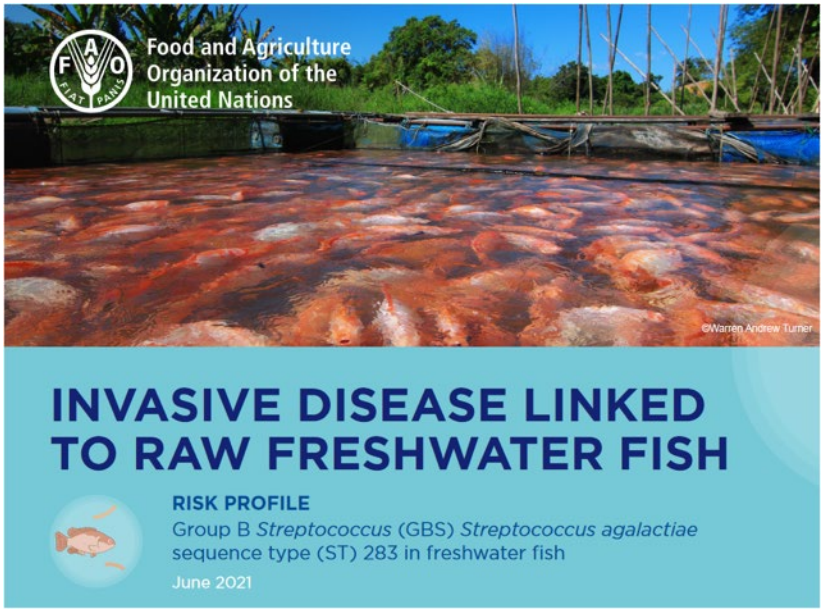


https://cge.cbs.dtu.dk/services/PathogenFinder/



https://cge.cbs.dtu.dk/cgi-bin/webface.fcgi?jobid=6107946F000009F219F0D4A7

MLST: *Streptococcus agalactiae* sequence type (ST) 283



http://www.fao.org/3/cb4901en/cb4901en.pdf

Clinical Infectious Diseases
SUPPLEMENT ARTICLE

2015 Epidemic of Severe *Streptococcus agalactiae* Sequence Type 283 Infections in Singapore Associated With the Consumption of Raw Freshwater Fish: A Detailed Analysis of Clinical, Epidemiological, and Bacterial Sequencing Data

Shirin Kalimuddin,[1,a] Swaine L. Chen,[2,3,a] Cindy T. K. Lim,[4,a] Tse Hsien Koh,[5] Thean Yen Tan,[6] Michelle Kam,[7] Christopher W. Wong,[3] Kurosh S. Mehershahi,[2] Man Ling Chau,[8] Lee Ching Ng,[8] Wen Ying Tang,[8] Hishamuddin Badaruddin,[10] Jeanette Teo,[11] Anucha Apisarnthanarak,[12] Nuntra Suwantarat,[12,13] Margaret Ip,[14] Matthew T. G. Holden,[15] Li Yang Hsu,[2,4] and Timothy Barkham[8];

PLOS NEGLECTED TROPICAL DISEASES

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

One hypervirulent clone, sequence type 283, accounts for a large proportion of invasive *Streptococcus agalactiae* isolated from humans and diseased tilapia in Southeast Asia

Timothy Barkham, Ruth N. Zadoks, Mohammad Noor Amal Azmai, Stephen Baker, Vu Thi Ngoc Bich, Victoria Chalker, Man Ling Chau, David Dance, Rama Narayana Deepak, H. Rogier van Doorn, Ramona A. Gutierrez, Mark A. Holmes, Lan Nguyen Phu Huong, [...], Swaine L. Chen [view all]

Published: June 27, 2019 • https://doi.org/10.1371/journal.pntd.0007421

Molecular serotyping on selected strains (GBS)

BLAST-based approach
(input = Fasta: assembled draft or complete genome)

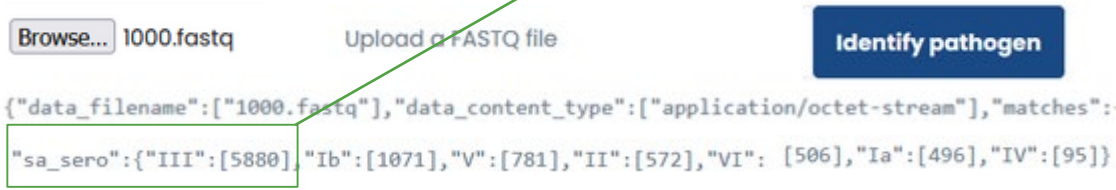
https://github.com/swainechen/GBS-SBG

```
# Name   Serotype
NF_3     GBS-SBG:III-4
# Name   Serotype
UPM_3    GBS-SBG:III-4
```

SAME RESULTS

Diagnostic k-mer-based approach
(input = Fastq > 400 read sequences - Assembly-Free and Real-Time)

In development by WorldFish, UQ and Wilderlab



Thank You

WorldFish

This work was undertaken as part of

CGIAR RESEARCH PROGRAM ON Fish Led by WorldFish

CGIAR Platform for Big Data in Agriculture

Inspire Challenge

In partnership with

The University Of Queensland AUSTRALIA

WILDERLAB

Mahidol University BIOTEC-NSTDA

GeneSEQ NEXT GENERATION SEQUENCING