Drinking Water Protection Conference 2023

From the source to the last flowing tap

Illustrating the use of metagenomic tools for characterising bacteria in drinking water: Case studies from working with Councils

Presented by
William Taylor

water NEW ZEALAND

The New Zealand Water & Wastes Association Waiora Aotearoa

Water is a National Security Issue

HARVARD LAW SCHOOL
NATIONAL SECURITY JOURNAL

Water Scarcity: The Most Understated Global Security Risk

by Major David J. Stuckenberg | May 18, 2018 | Features, Online Edition

DAVOS 2023

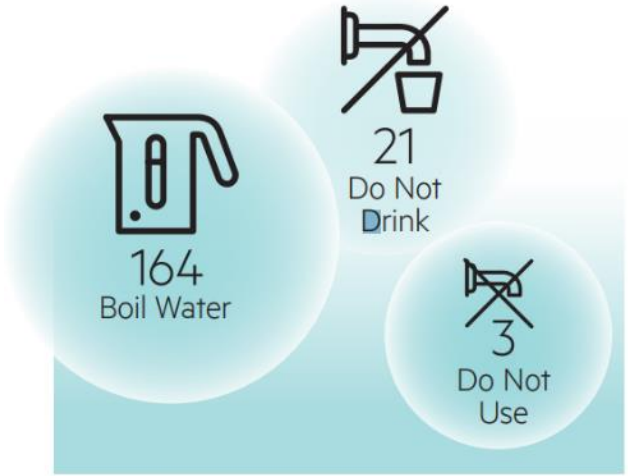
WORLD ECONOMIC FORUM

Water security is a national security issue: What's needed now

Feb 8, 2023

Consumer advisories 2022

- 911 notifications in 2022
  - 387 level above MAV
    - Microbial and chemical
  - 495 ‘other risks’
  - 23 supply interruptions
  - 6 complaints/concerns
  - 75 supplies *E. coli* >MAV
  - 45 Schools and a NZDF facility, *E. coli* >MAV



https://www.taumataarowai.govt.nz/assets/Uploads/Governance-docs/Drinking-Water-Regulation-Report-2022.pdf

Faecal Contamination: *E. coli*

Theodor Escherich, 1895

EXAMINATION OF WATER.

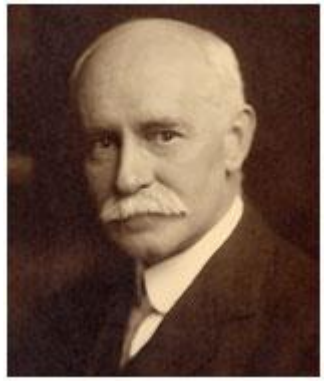
(CHEMICAL AND BACTERIOLOGICAL.)

JOHN WILEY & SONS.
LONDON: CHAPMAN & HALL, LIMITED.
1899.

BY

WILLIAM P. MASON,

PROFESSOR OF CHEMISTRY, RENSSELAER POLYTECHNIC INSTITUTE;
Member of the American Philosophical Society, the American Chemical Society, the American Public Health Association, the American Water-Works Association, the New England Water-Works Association, the Franklin Institute, etc., etc.



theria bacillus, we shall do well to avoid such expressions as contain 'index of fæcal pollution.' It is an assumption to say that *B. coli communis* does not occur in abundance in organic matters other than animal excreta." *

It is certainly the author's experience that the "colon group" is widely distributed, he having found it in waters that a "sanitary survey" would unquestionably pronounce pure; but it cannot be denied that its persistent presence in large numbers is an indication of pollution that must not be overlooked;

Science for Communities
He Pūtaiao, He Tāngata

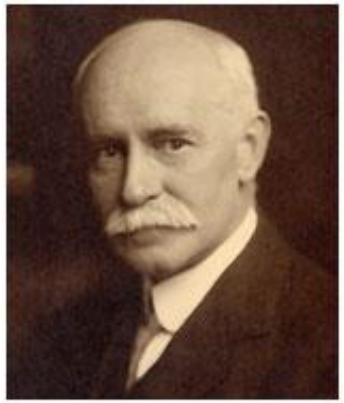
TAUMATA AROWAI

Water Services (Drinking Water Standards for New Zealand) Regulations 2022

Table 1

Maximum acceptable values for microbiological determinands

Determinand	Maximum acceptable value
Escherichia coli	Less than 1 in 100 mL of sample
Total pathogenic protozoa	Less than 1 verified infectious (oo)cyst per 100 L of sample



DRINKING WATER QUALITY ASSURANCE RULES

2022

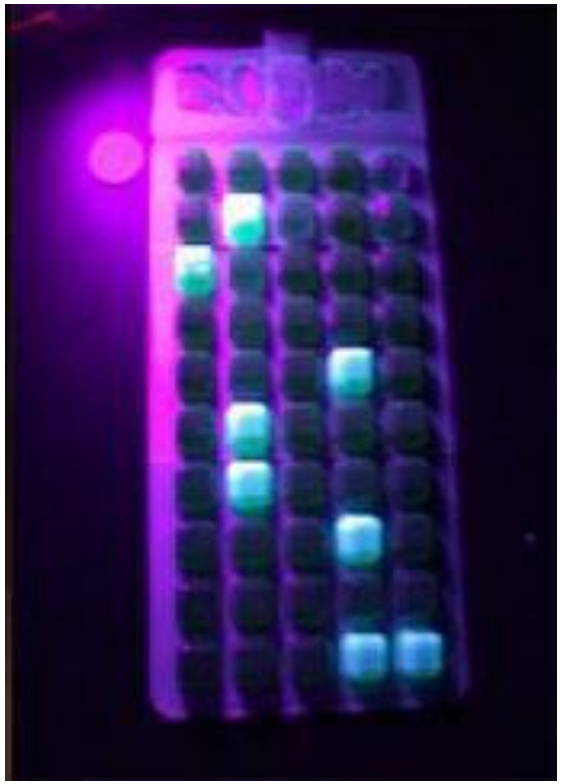
Released 25 July 2022

Parameter
E. coli, total coliforms

Colilert Test

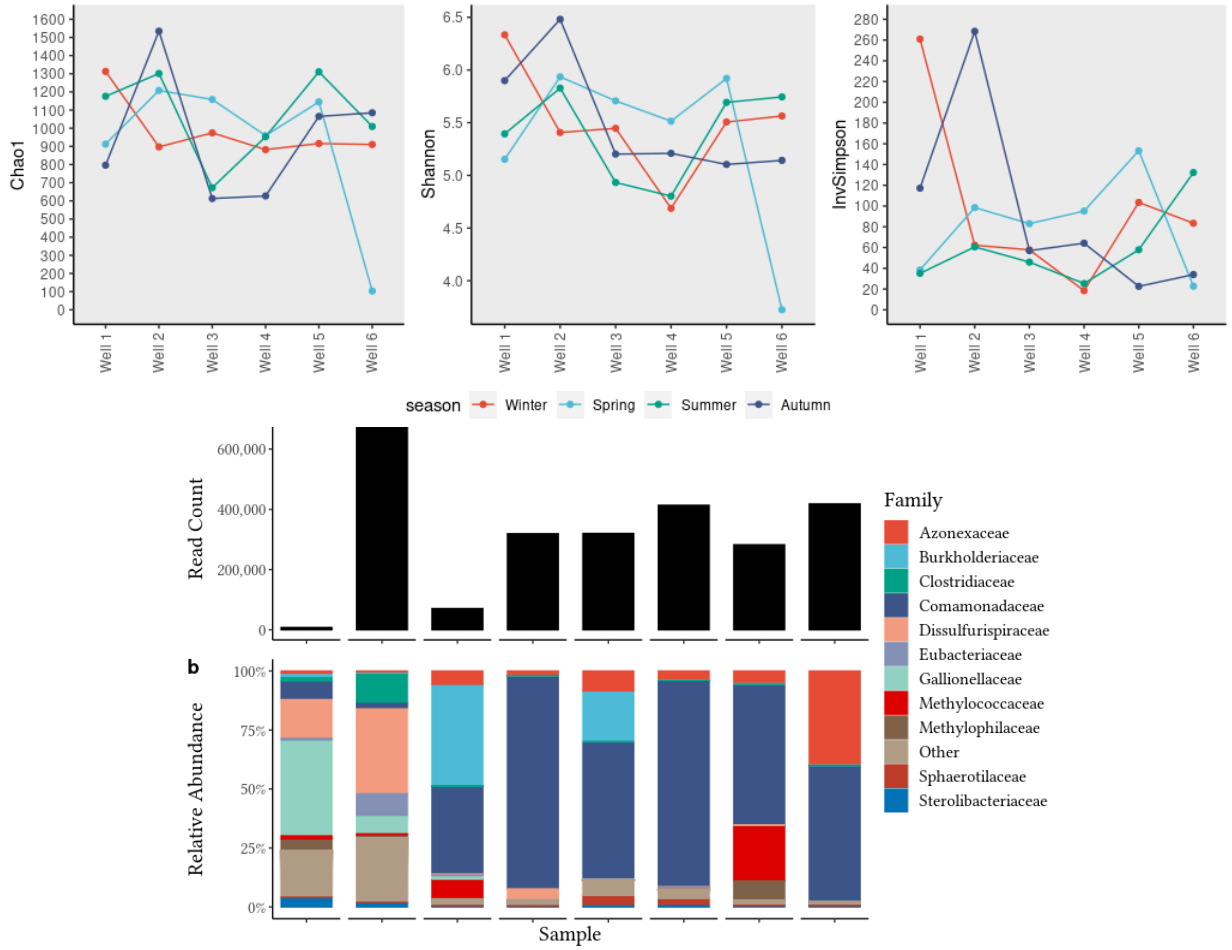
Yellow = Coliform

Glow blue = E. coli

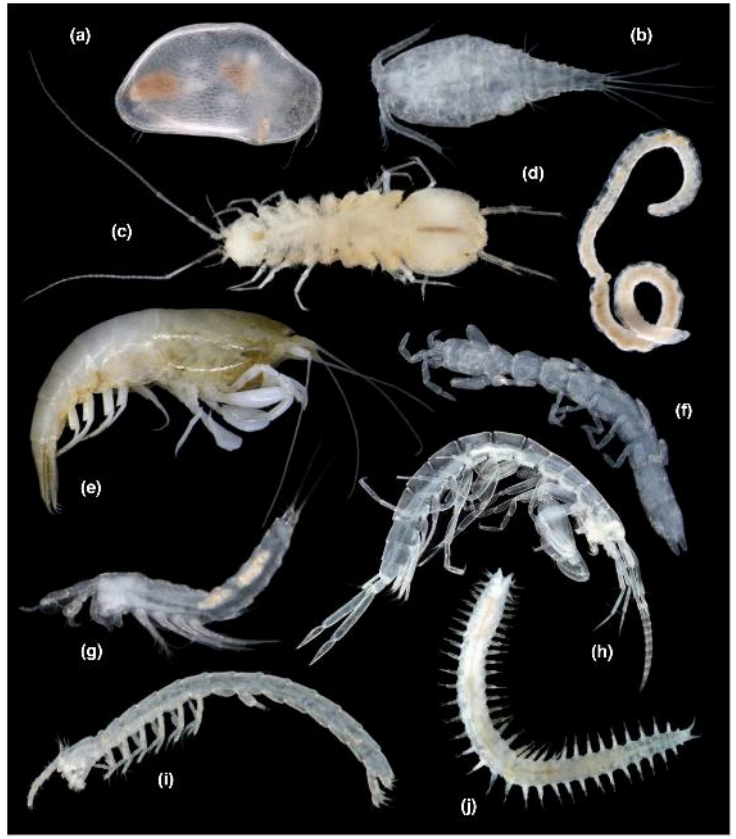


https://www.idexx.com/en/water/water-products-services/colilert/

Groundwater is not sterile



Diversity and families in groundwater bores over time



Stygofauna found in Groundwater systems

Human Microbiome

- 10-100 trillion microbes on every person
  - 95% in gut
  - Gut microbiota up to 2 KG
- 100 microbial genes for each human gene
- >10,000 species

https://cosmosmagazine.com/science/biology/how-many-genes-in-the-human-microbiome/

Kwork et al.; Face touching: A frequent habit that has implications for hand hygiene. American Journal of Infection Control 2015
WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care.

Human Microbiome

- 10-100 trillion microbes on every person
  - 95% in gut
  - Gut microbiota up to 2 KG
- 100 microbial genes for each human gene
- >10,000 species
- Humans touch their face 23 times an hour
  - 44% involved touching a mucous membrane
  - Human fingertips transfer hundreds of bacteria
    - ~1500 cells/cm^2

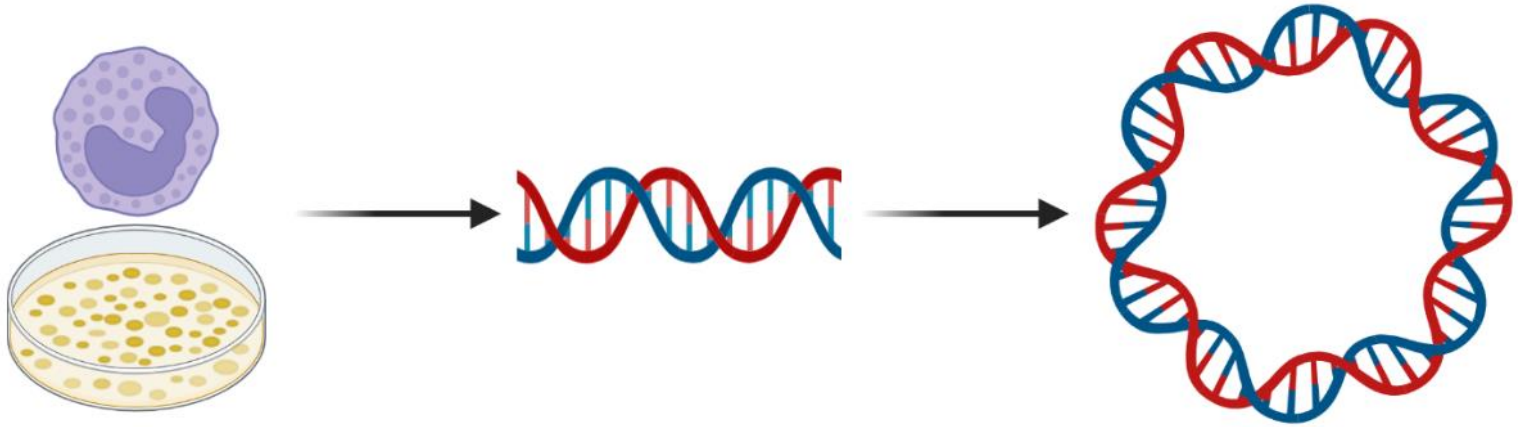
https://cosmosmagazine.com/science/biology/how-many-genes-in-the-human-microbiome/

Kwork et al.; Face touching: A frequent habit that has implications for hand hygiene. American Journal of Infection Control 2015
WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care.

Metagenomics vs Genomics

Genomics

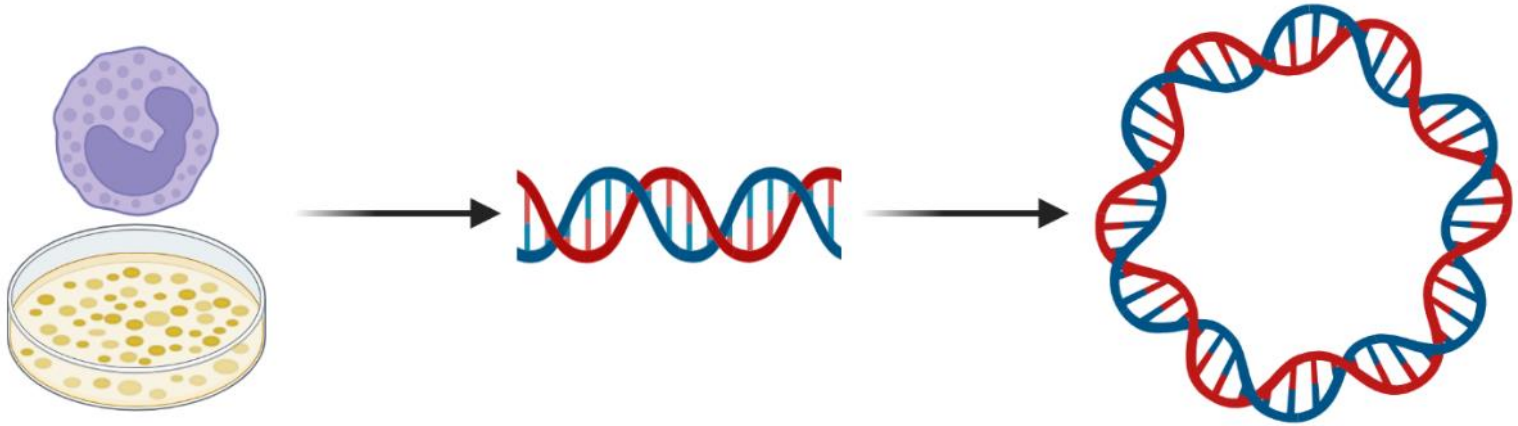
Single organism



Metagenomics vs Genomics

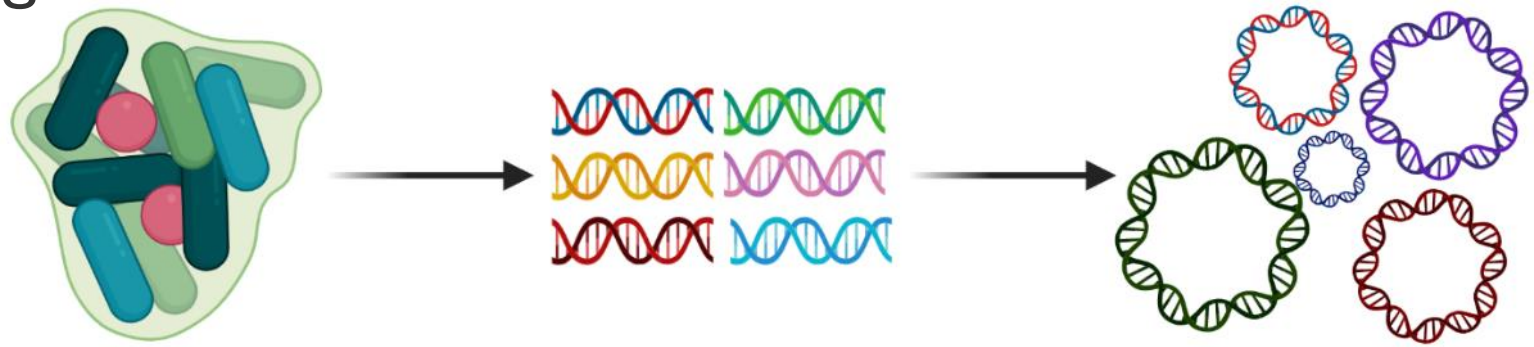
Genomics

Single organism

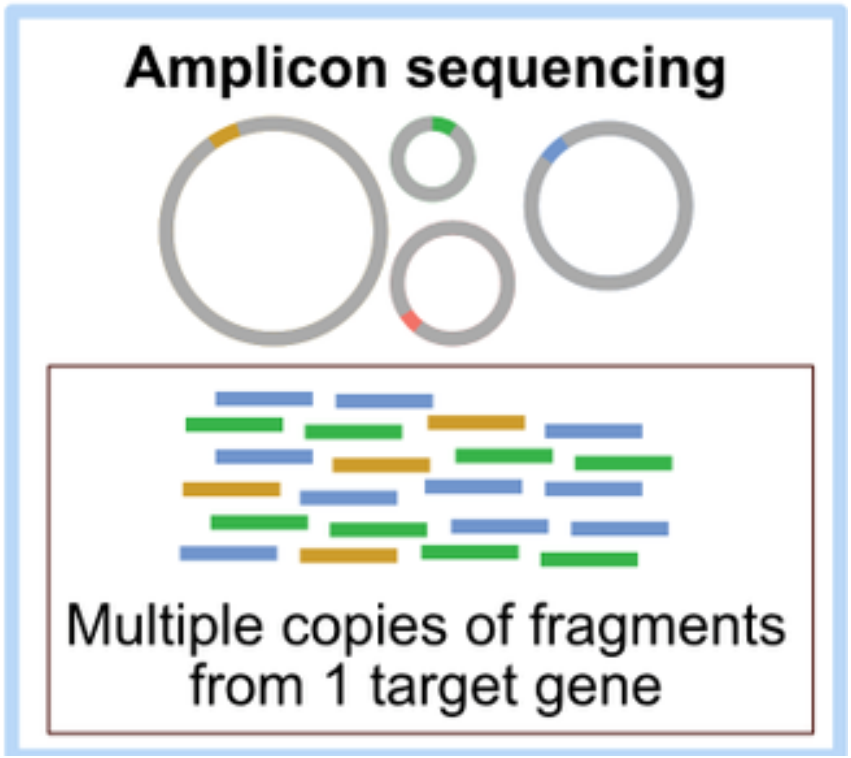


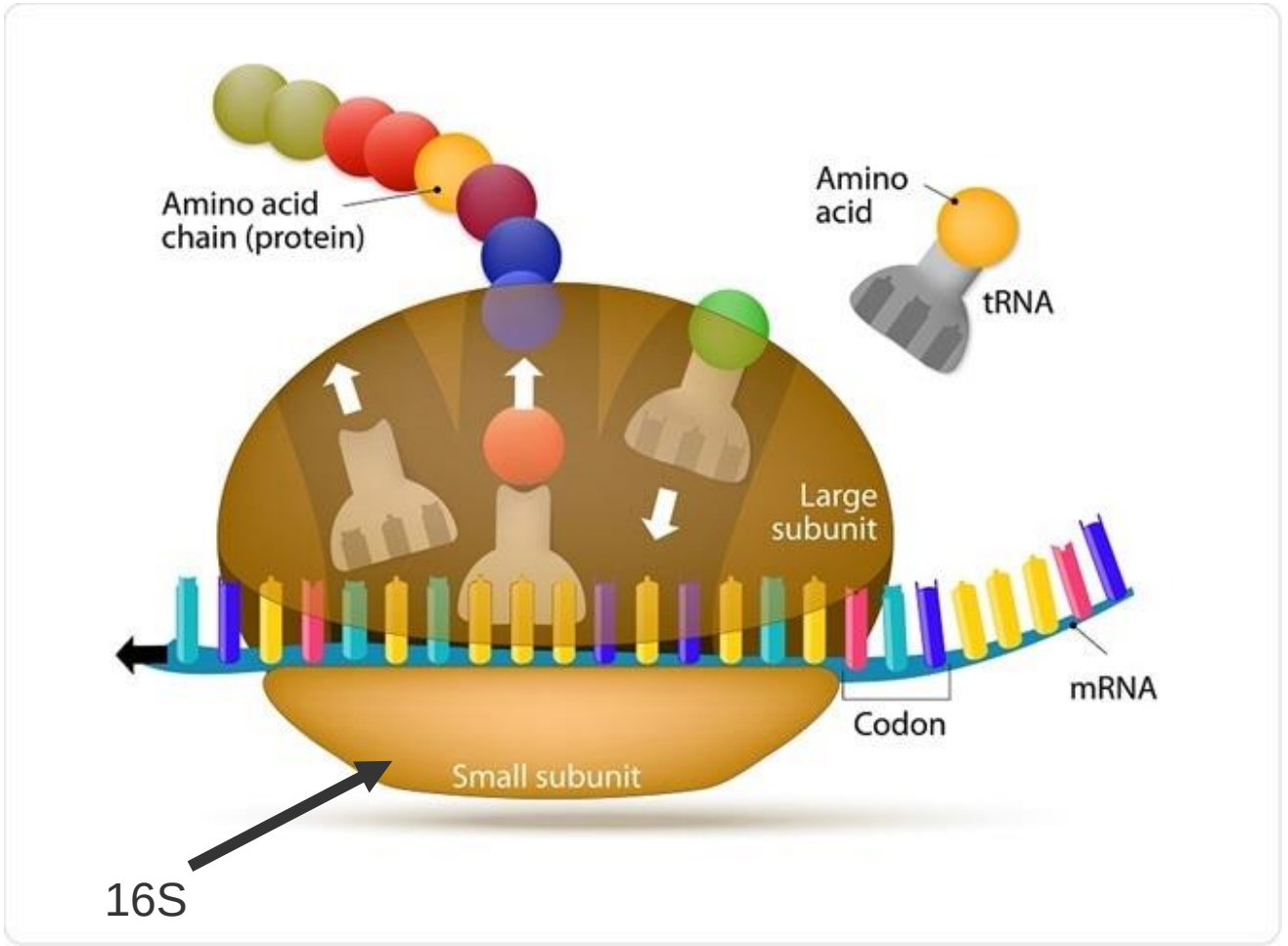
Metagenomics

Many organisms



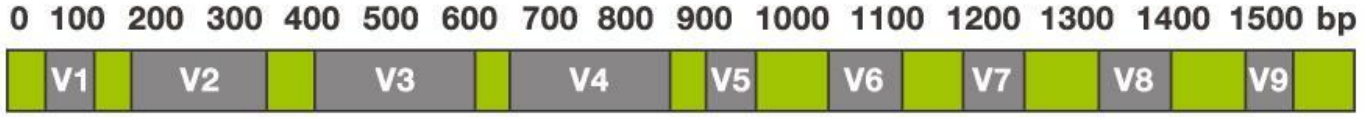
Amplicon Metagenomics





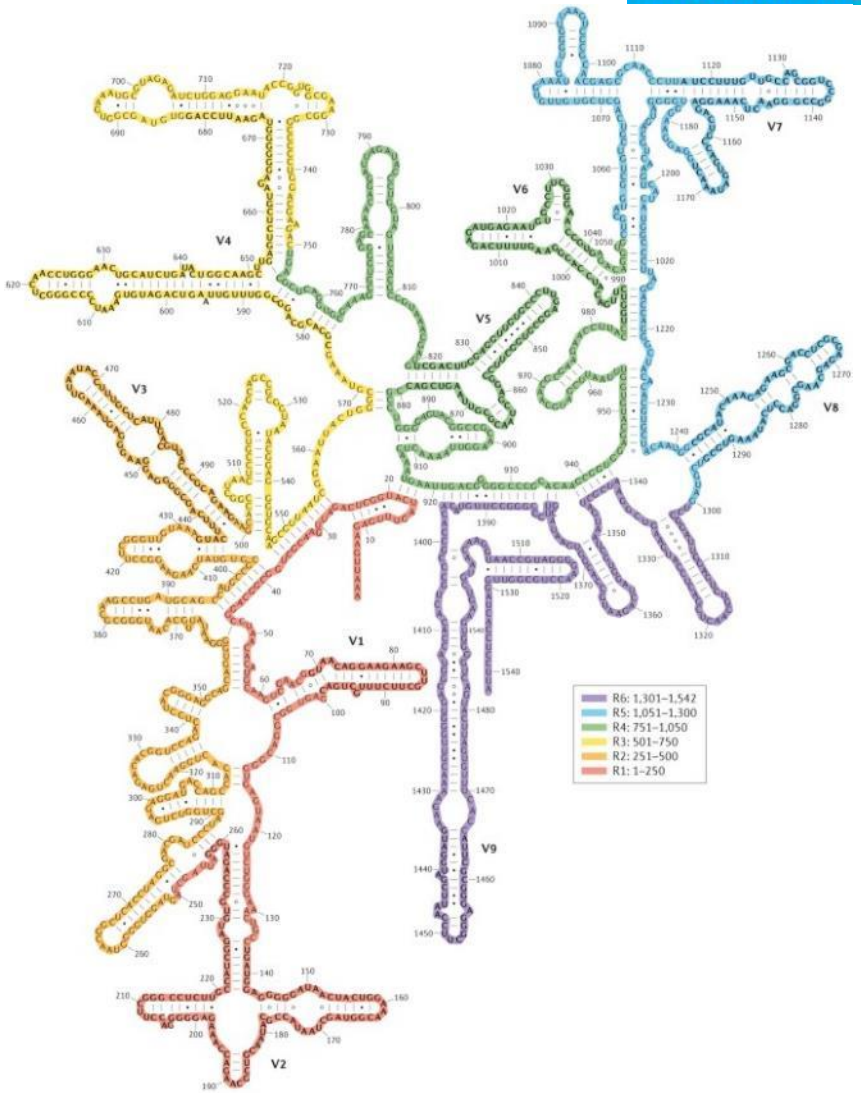
https://www.news-medical.net/life-sciences/Ribosome-Structure.aspx

16S Ribosomal RNA



ATCG….

…ATCG

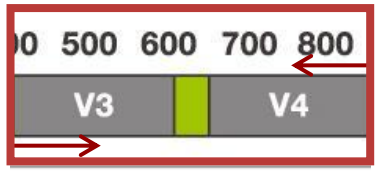


Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences, Yarza et al. 2014, Nature Reviews Microbiology

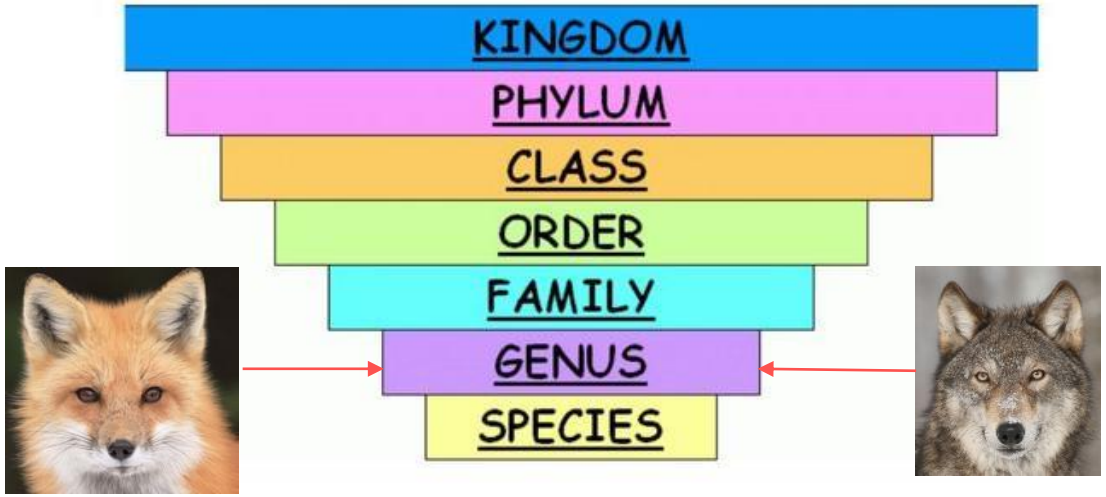
Nature Reviews | Microbiology

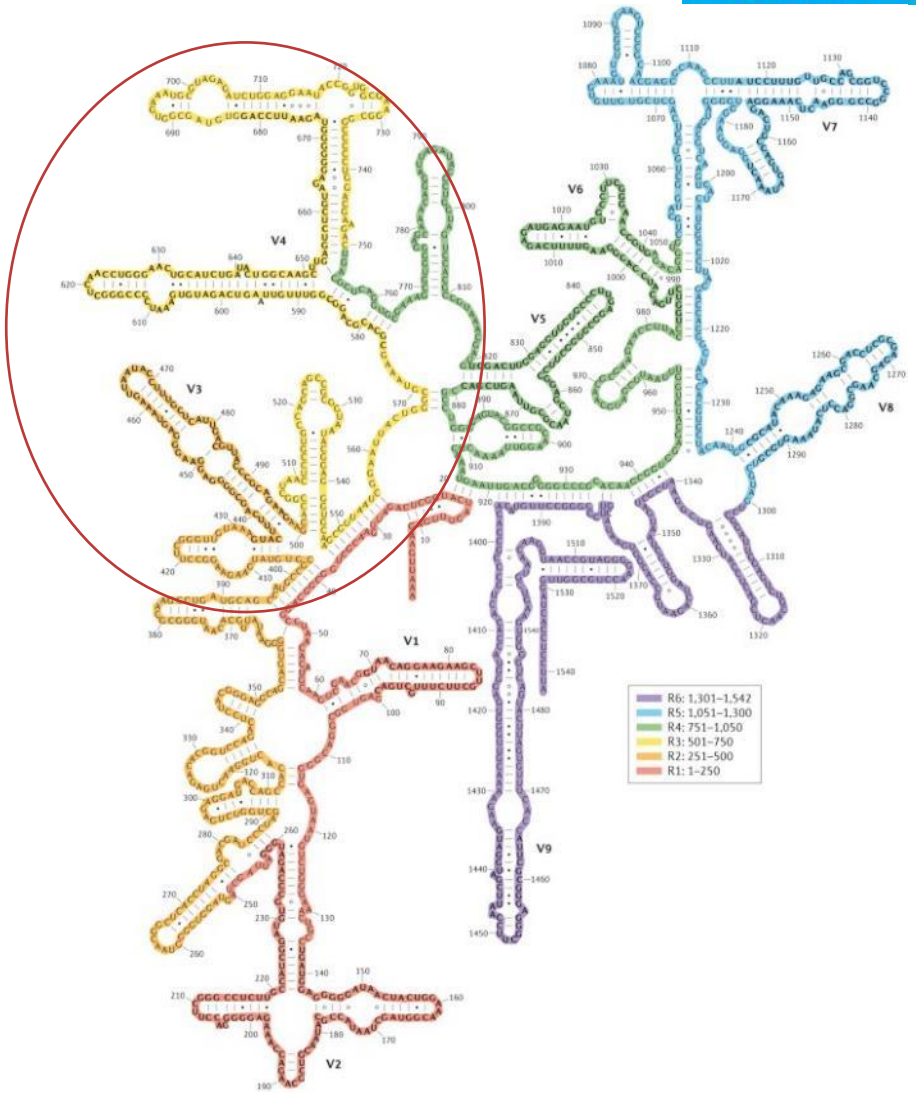
16S Ribosomal RNA

~450 bp



Illumina



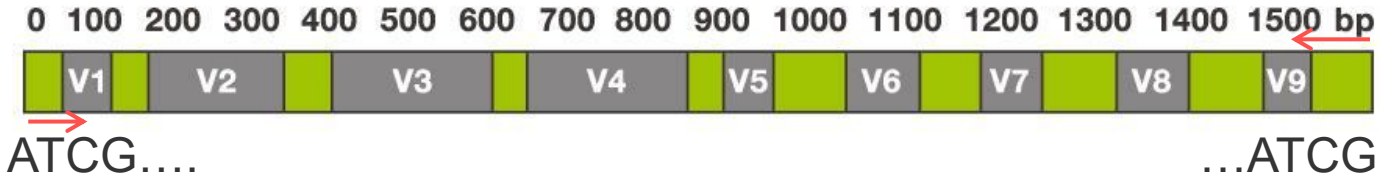


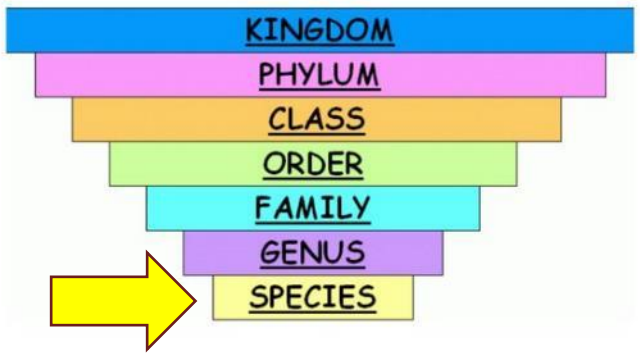
Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences, Yarza et al. 2014, Nature Reviews Microbiology

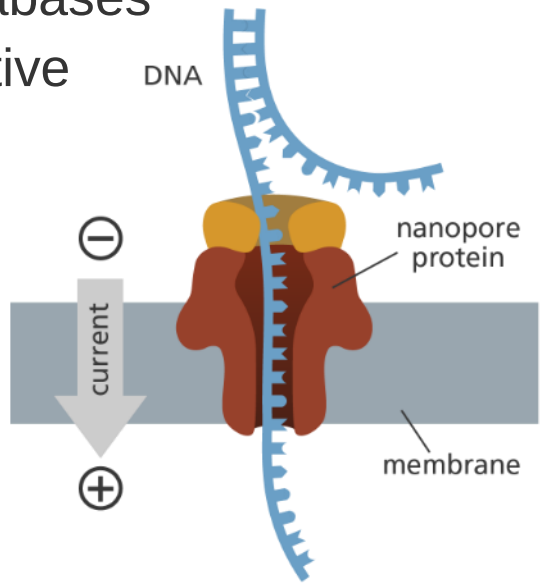
Nature Reviews | Microbiology

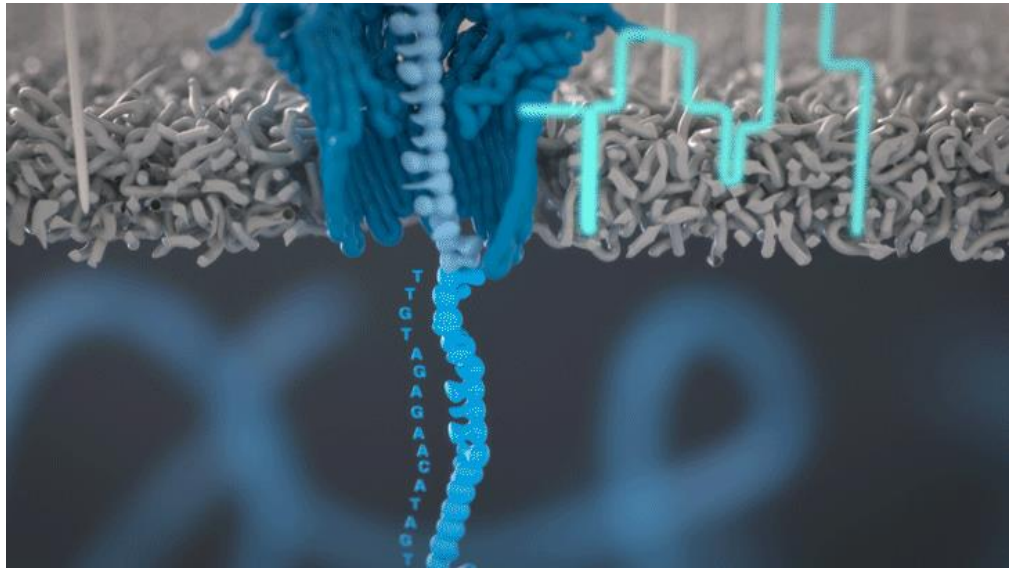
Nanopore Full Length 16S Ribosomal RNA

- Pros:
  - Large query sequence
  - Lower taxonomic level
  - Well established databases
  - Rapid and cost effective
  - Small footprint









https://nanoporetech.com/support/how-it-works

Short Reads vs Long Reads

- Do we have a potentially disease-causing organism in our sample?

Short Reads vs Long Reads

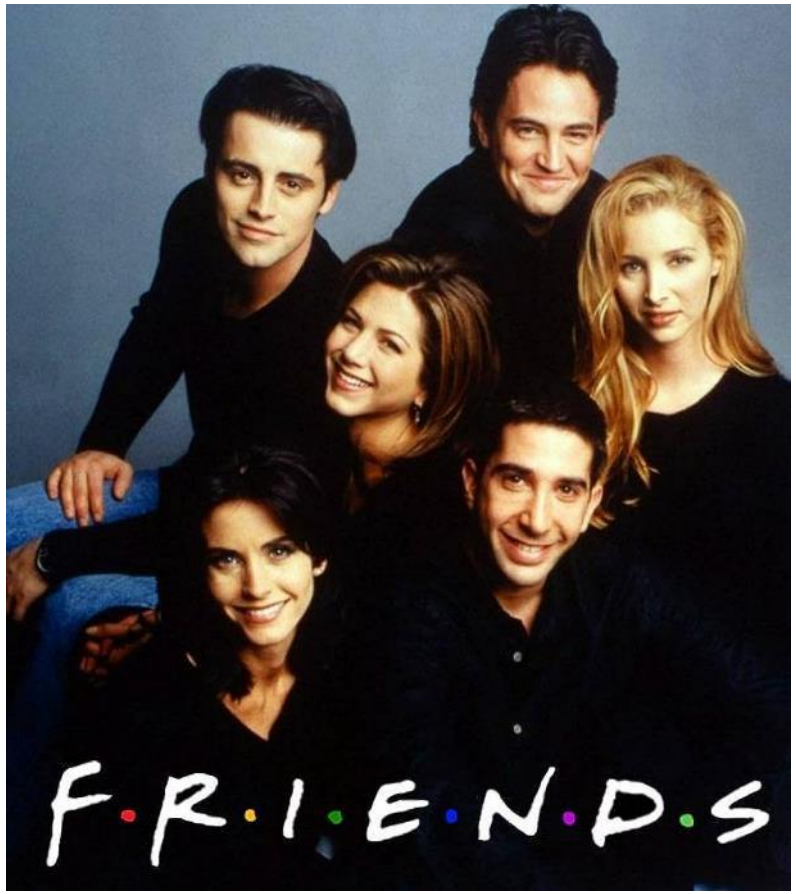
- Do we have a potentially disease-causing organism in our sample?

- Analogy:
  - Portion of face = amplicon
  - IMDB as database

Short Reads vs Long Reads

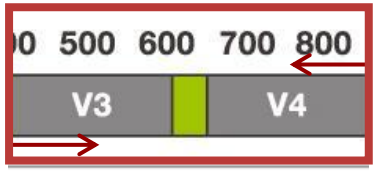
- Do we have a potentially disease-causing organism in our sample?

- Analogy:
  - Portion of face = amplicon
  - IMDB as database
  - Cast of Friends in place of disease-causing organism



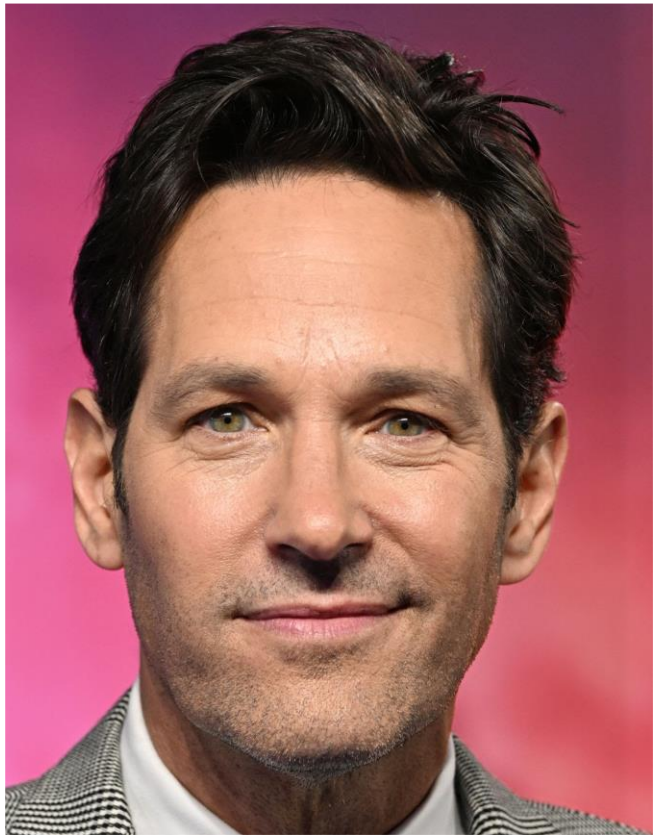
Short Reads vs Long Reads

~450 bases

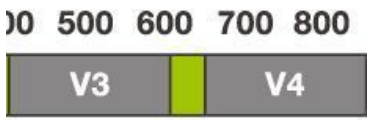


Short read (Illumina)

Short Reads vs Long Reads



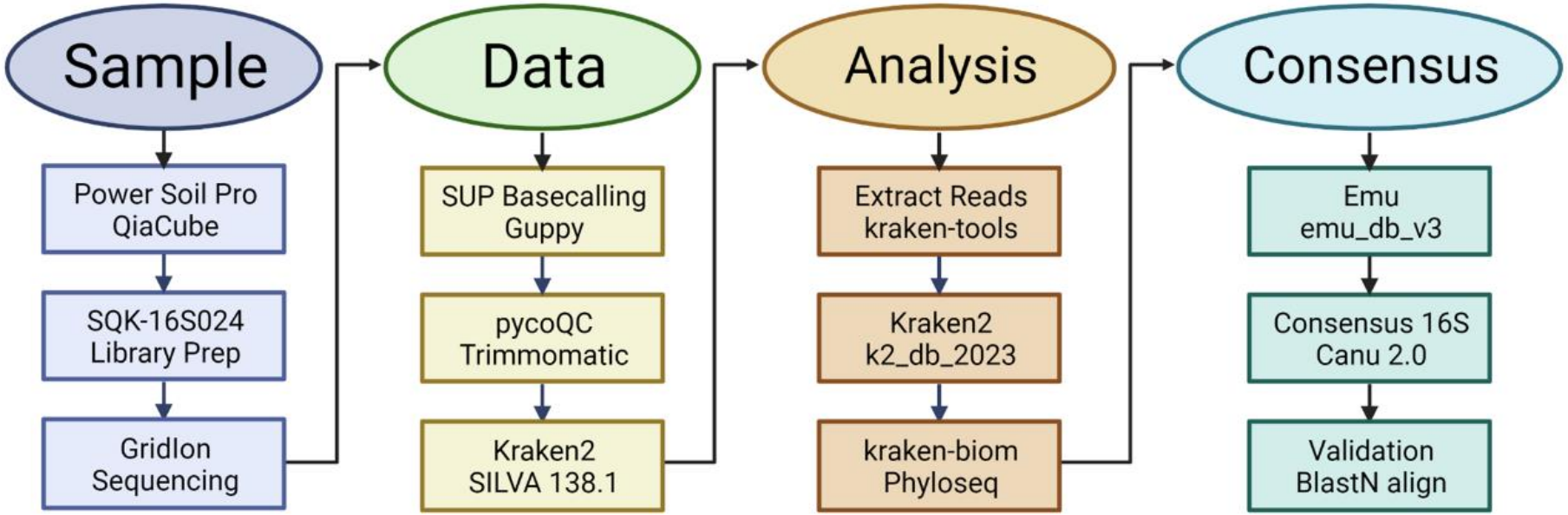
~450 bases



Short read (Illumina)

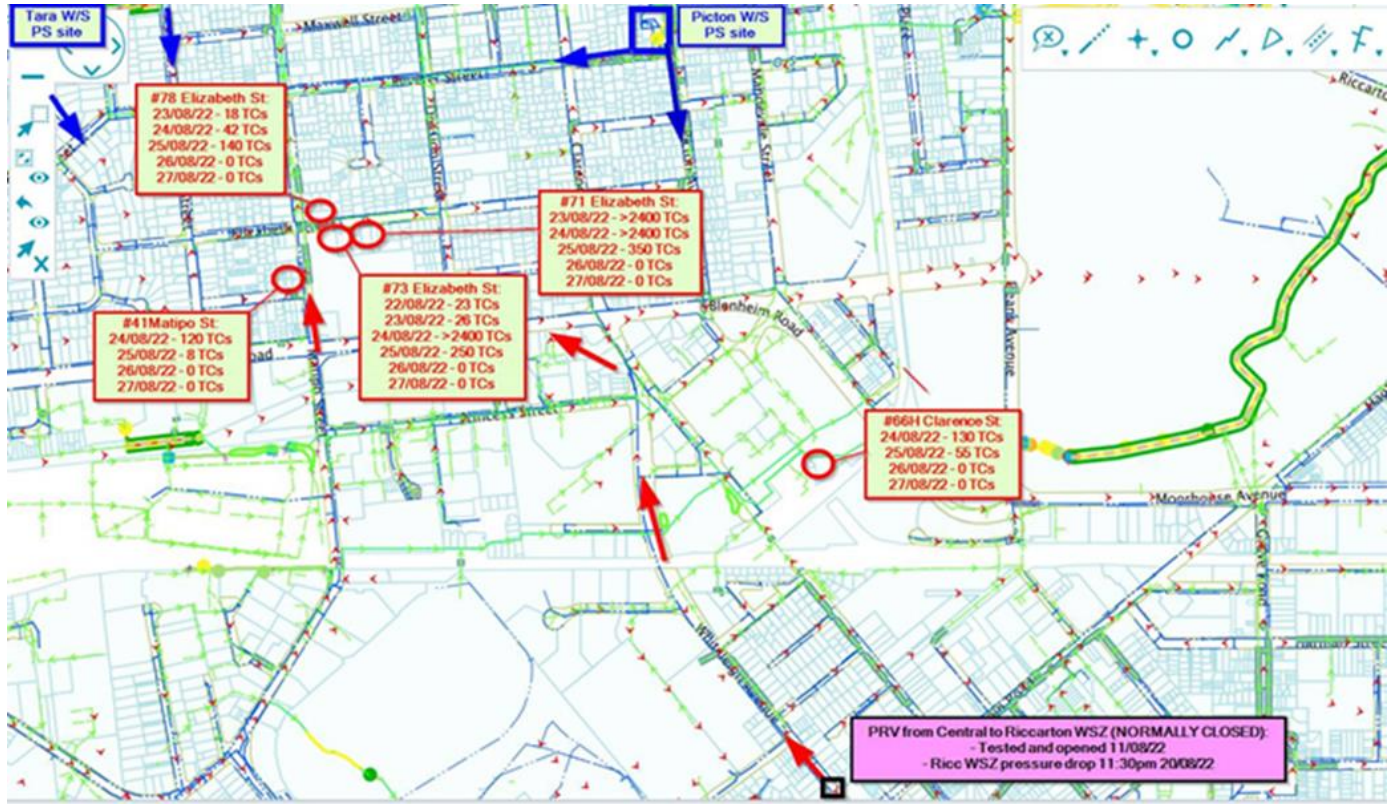
~1500 bases
Long read (Nanopore)

Analysis Pipeline

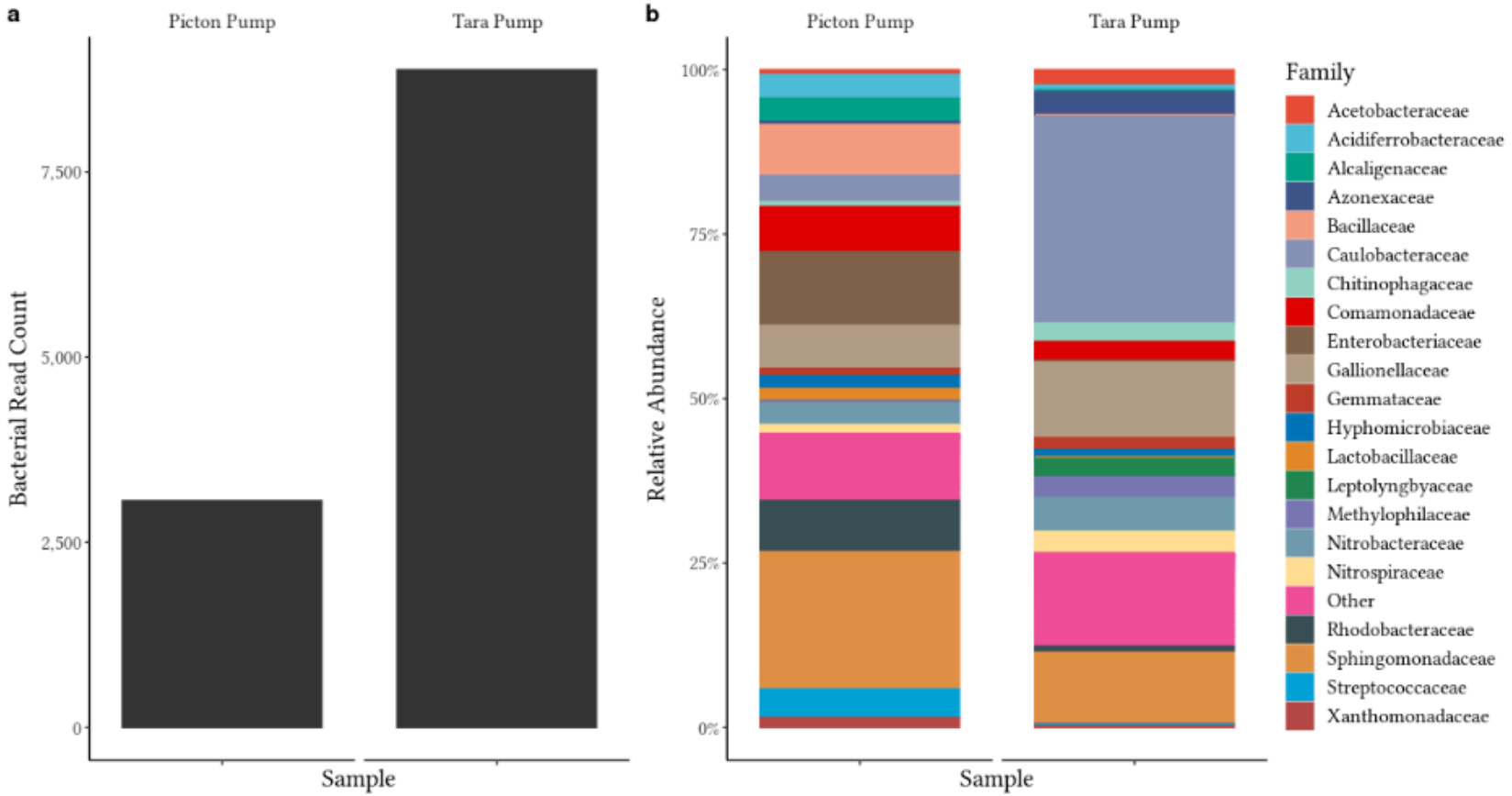


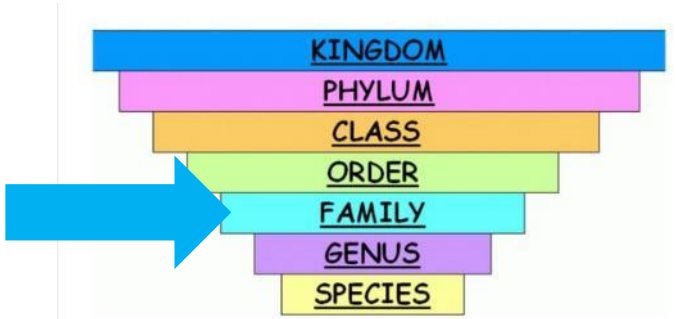
Case Study 1: Christchurch

- 23rd-24th of August
  - Sites around Riccarton
  - Total coliform >2400
  - Speculated pressure release valve was to blame
- 1L Samples taken at 12pm (prior) and 1-2 pm (after)

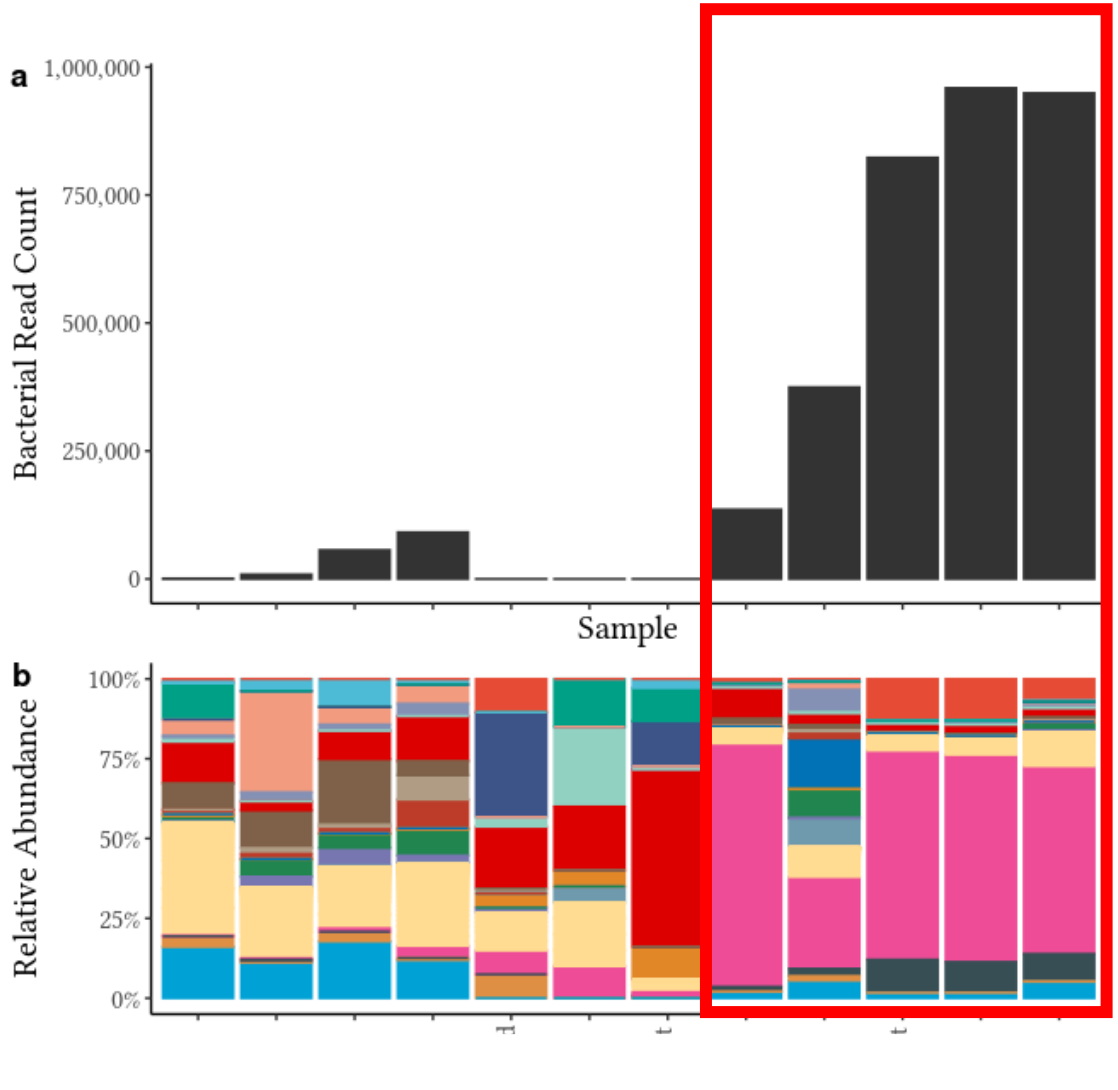


Source Waters: Pump Stations

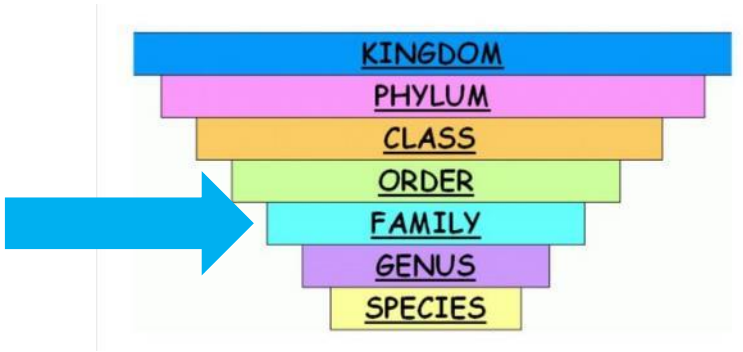




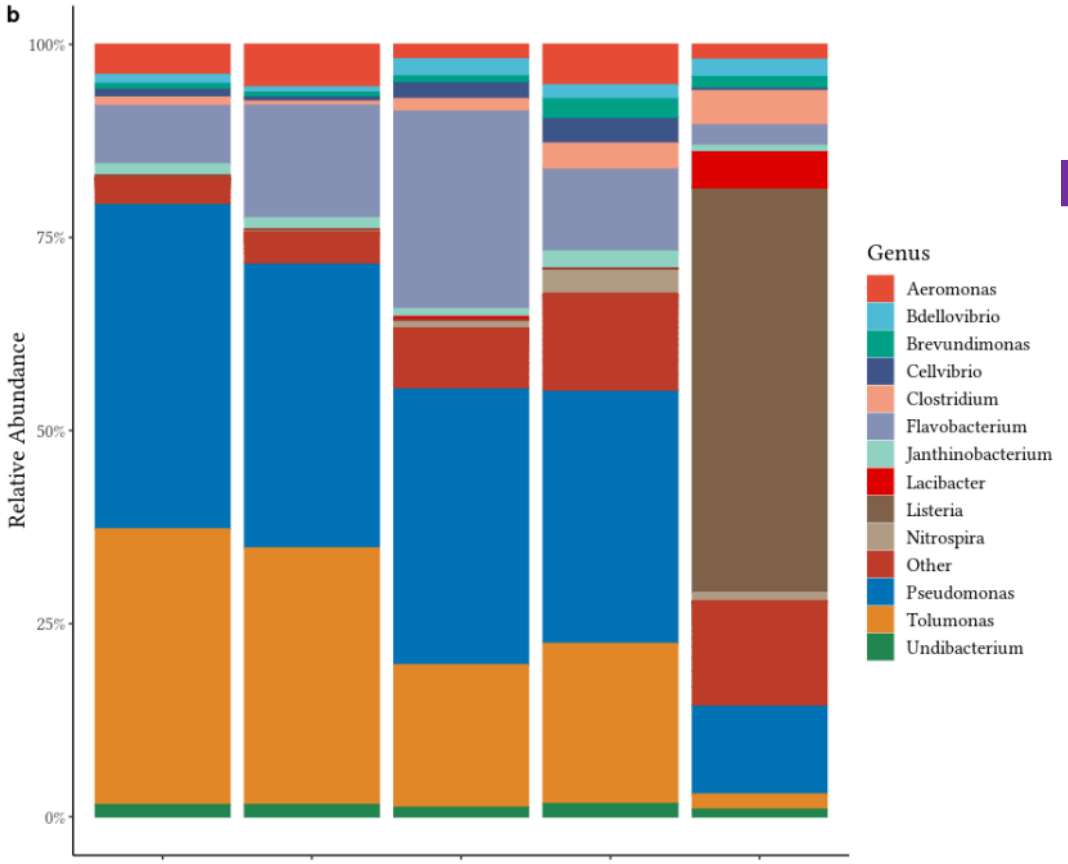
1,000,000 counts

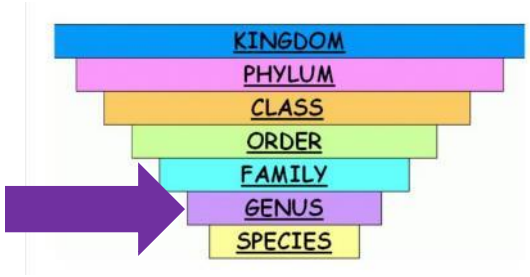


Family
- Aeromonadaceae
- Azonexaceae
- Bacillaceae
- Burkholderiaceae
- Caulobacteraceae
- Chitinophagaceae
- Clostridiaceae
- Comamonadaceae
- Gallionellaceae
- Gemmataceae
- Legionellaceae
- Listeriaceae
- Moraxellaceae
- Nitrobacteraceae
- Nitrospiraceae
- Opitutaceae
- Other
- Oxalobacteraceae
- Pseudomonadaceae
- Rhodobacteraceae
- Sphingomonadaceae

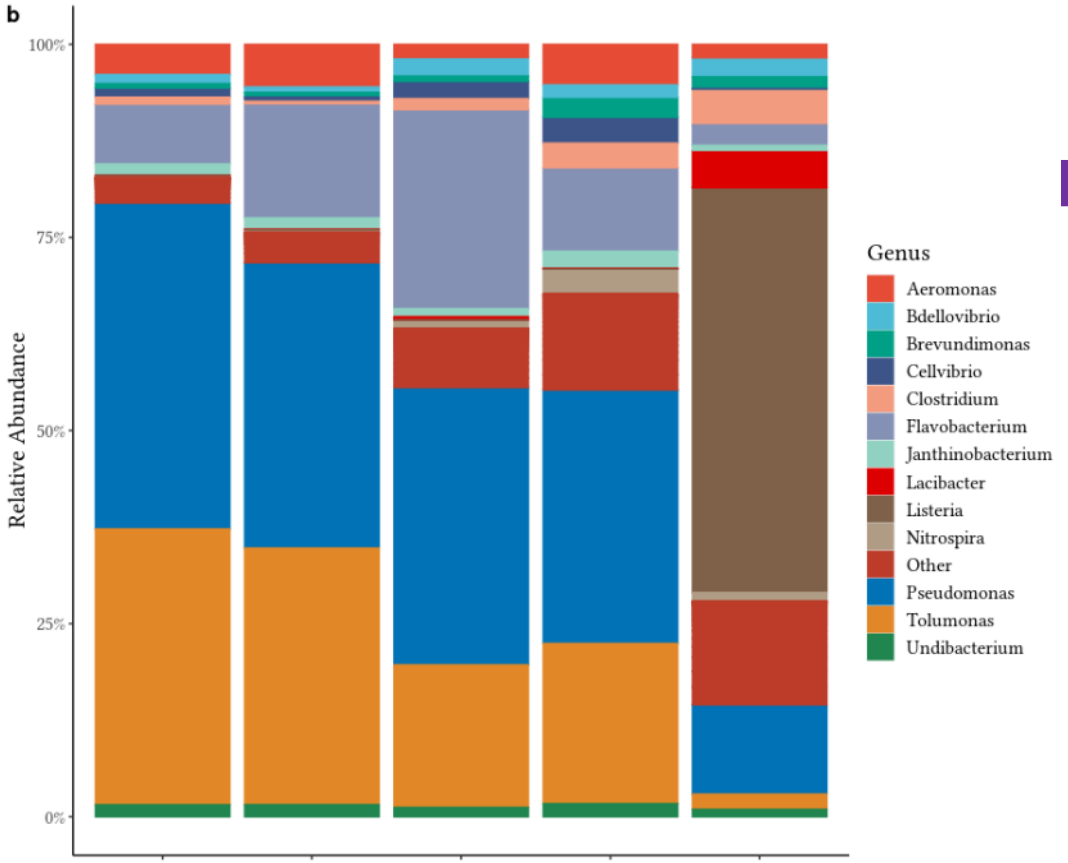


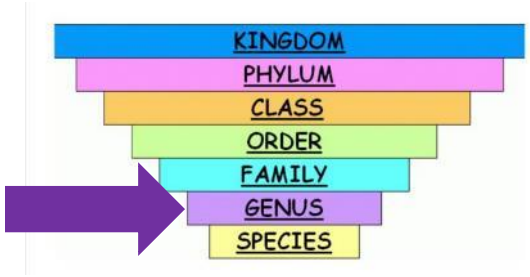
Samples with FAC<0.1 + TC

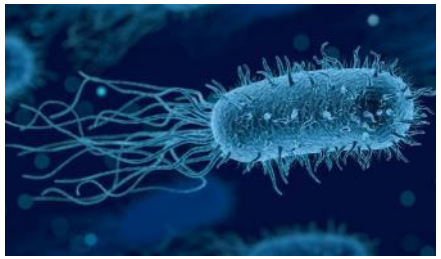




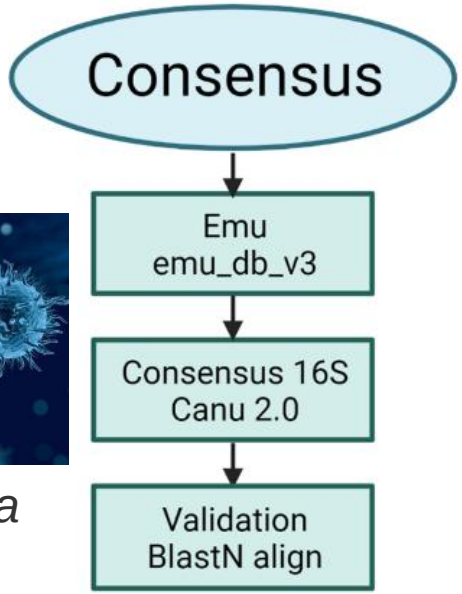
Samples with FAC<0.1 + TC

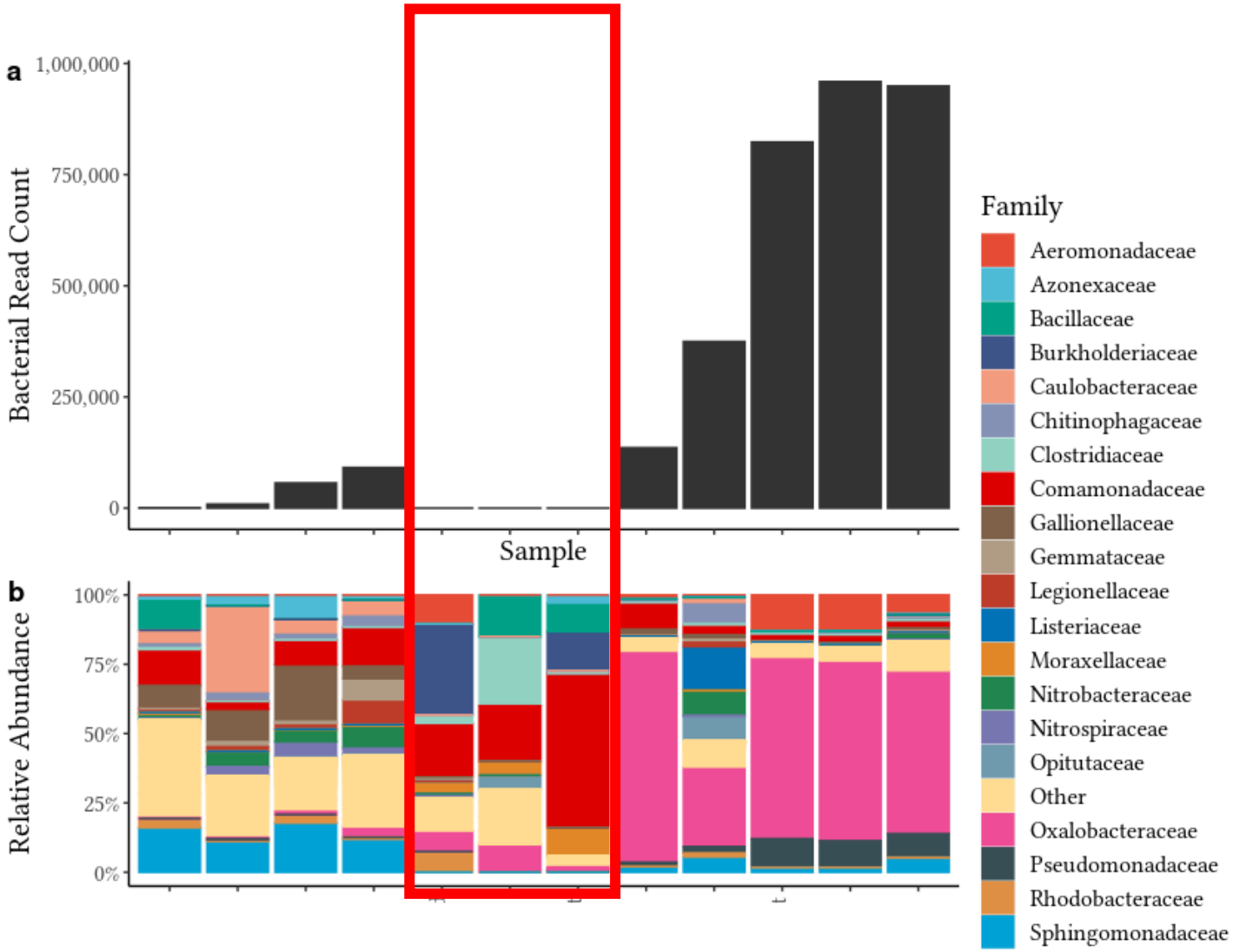


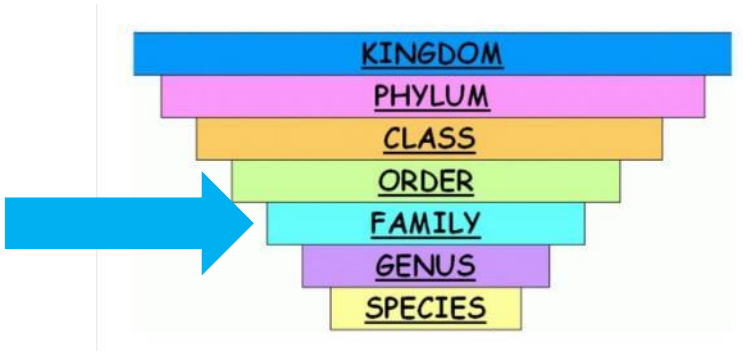




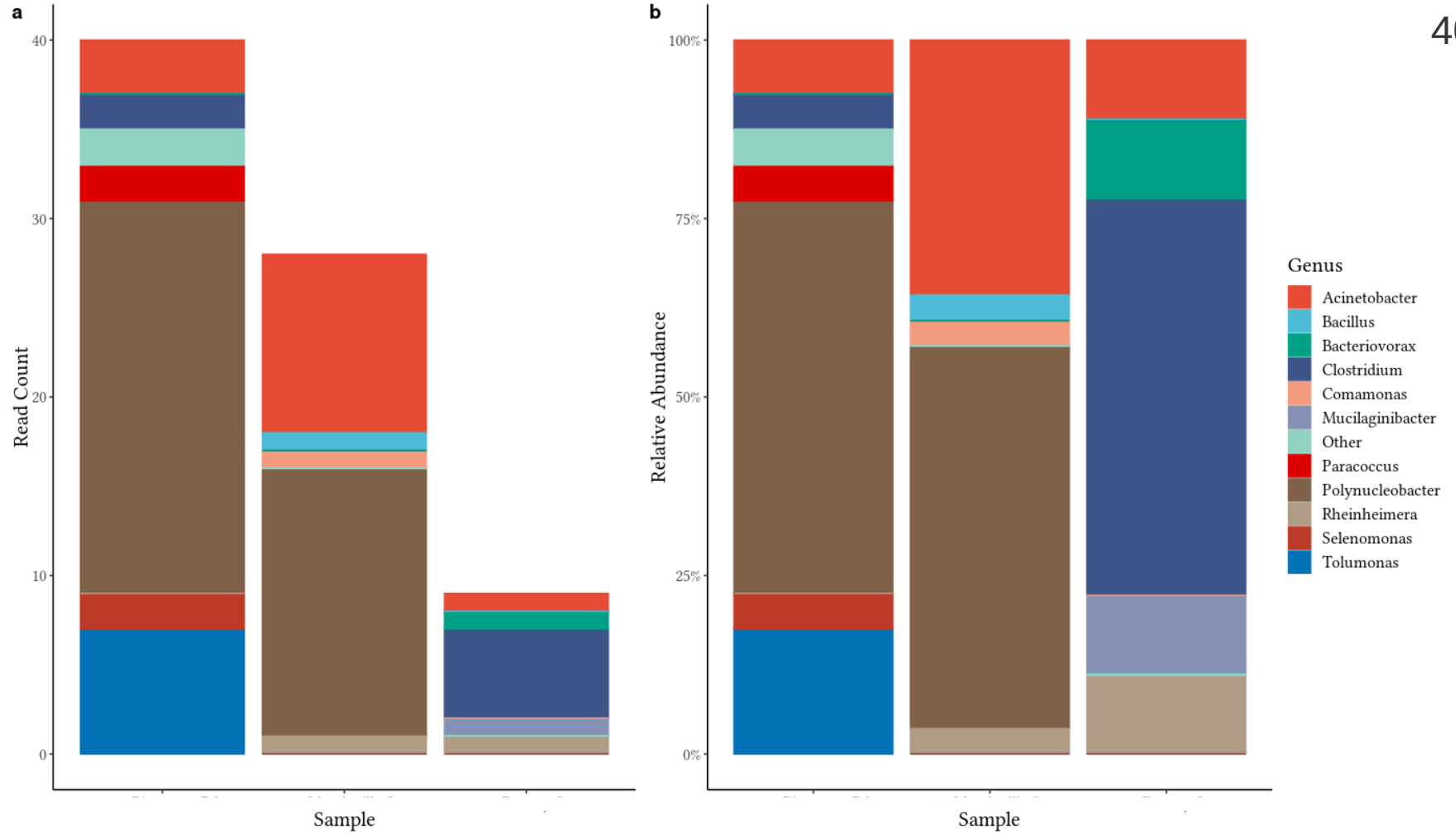
Listeria innocua




a
1,000,000
750,000
500,000
250,000
0
Bacterial Read Count
Sample
b
100%
75%
50%
25%
0%
Relative Abundance
Family
Aeromonadaceae
Azonexaceae
Bacillaceae
Burkholderiaceae
Caulobacteraceae
Chitinophagaceae
Clostridiaceae
Comamonadaceae
Gallionellaceae
Gemmataceae
Legionellaceae
Listeriaceae
Moraxellaceae
Nitrobacteraceae
Nitrospiraceae
Opitutaceae
Other
Oxalobacteraceae
Pseudomonadaceae
Rhodobacteraceae
Sphingomonadaceae


KINGDOM
PHYLUM
CLASS
ORDER
FAMILY
GENUS
SPECIES

Samples with FAC > 0.68



40 counts

	Site	Date/Time	Total Coliforms	FAC	Read Density	Pathogens	Faecal	Surface Water	Groundwater	Non-source water	Interpretations Conclusions
		14.10	<1	NA	1,400					-	
		14.58	<1	NA	11,036					-	
		13.53	<1	0.82	431						Chlorination destroyed microbial pop.
		14.24	<1	0.72	336						
		14.41	<1	0.68	95						
		12.12	250	<0.10	1,274,051						
		12.43	140	<0.10	1,190,670						
		12.24	350	<0.10	1,067,940						
		10.35	55	<0.10	467,442						
		13.00	8	<0.10	187,052						
		15.31	<1	<0.10	67,507						
		15.15	<1	<0.10	109,037						

Case Study 2: Birdlings Flat

- Samples from 5th and 6th March 2023
  - Total coliforms detected
  - Unchlorinated
  - Chlorination started
  - Black flecks of soft metallic material caught in filter post chlorination

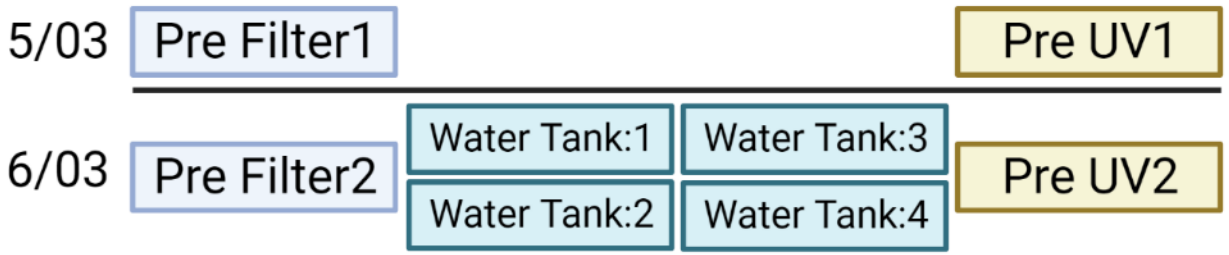


Figure 1. Locations of Samples Taken

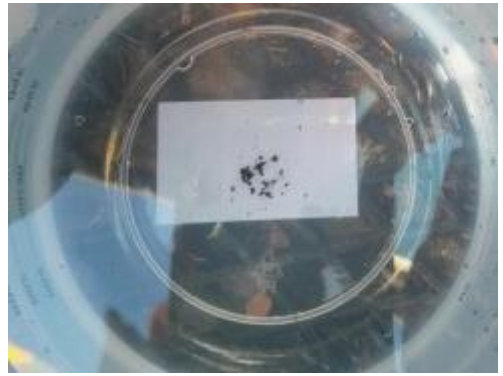
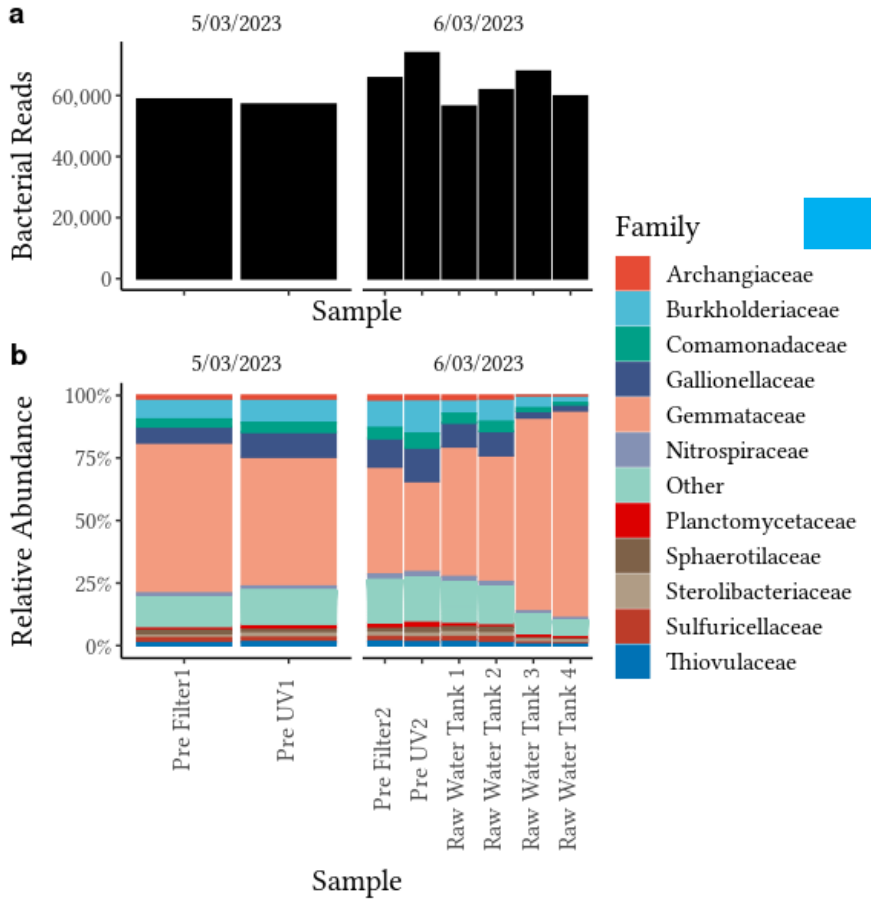
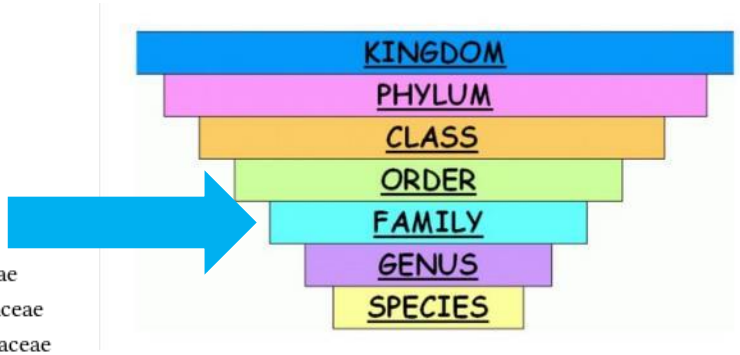


Figure 2. Black flecks found in filters

Samples Prior to Chlorination

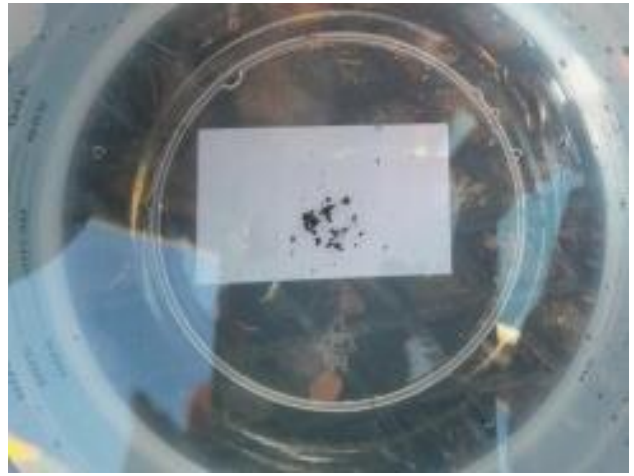
- Consistent microflora/read levels across samples
  - Slight difference in water tanks 3 & 4
- No pathogenic or faecal bacteria
- All identified bacteria were environmental and associated with groundwater environments

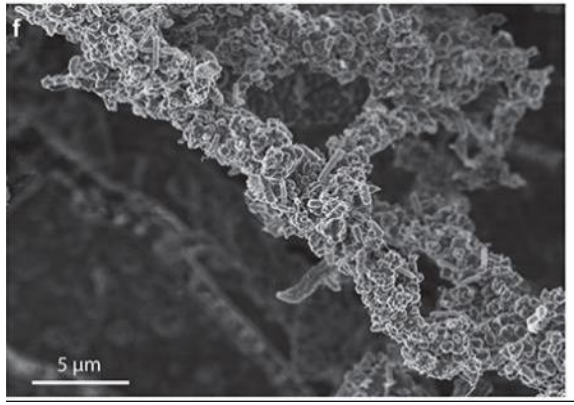


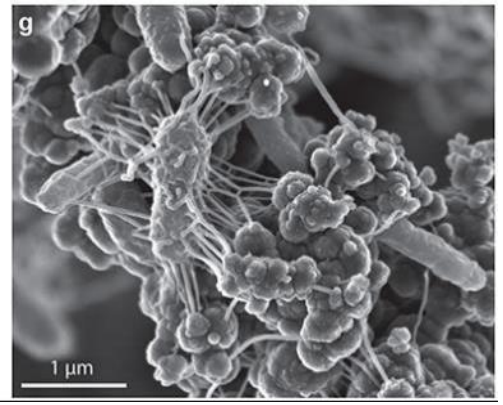


What caused the flecks?

- Black flecks?
  - High iron + manganese, both soluble in water
  - Bacteria (i.e., *Gallionella*, *Sideroxydans*) can sequester and concentrate these elements in their cells and biofilms
  - Chlorine caused it to precipitate

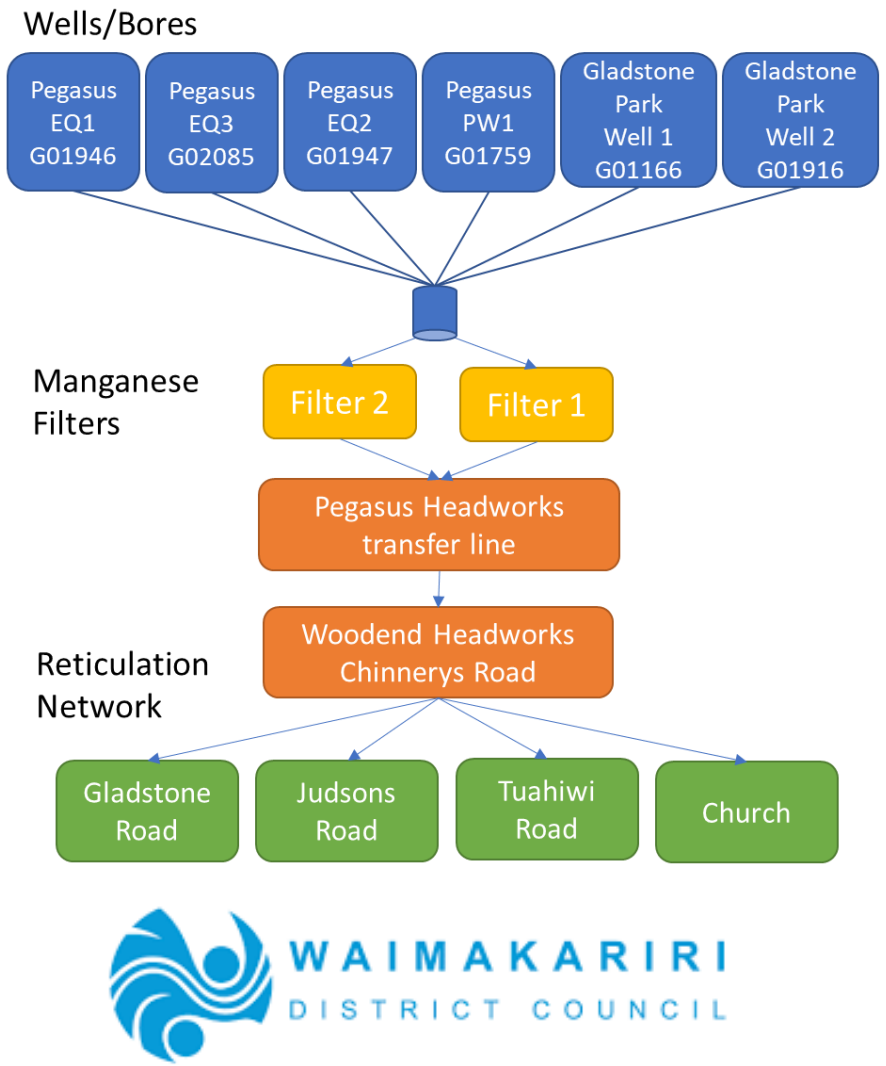




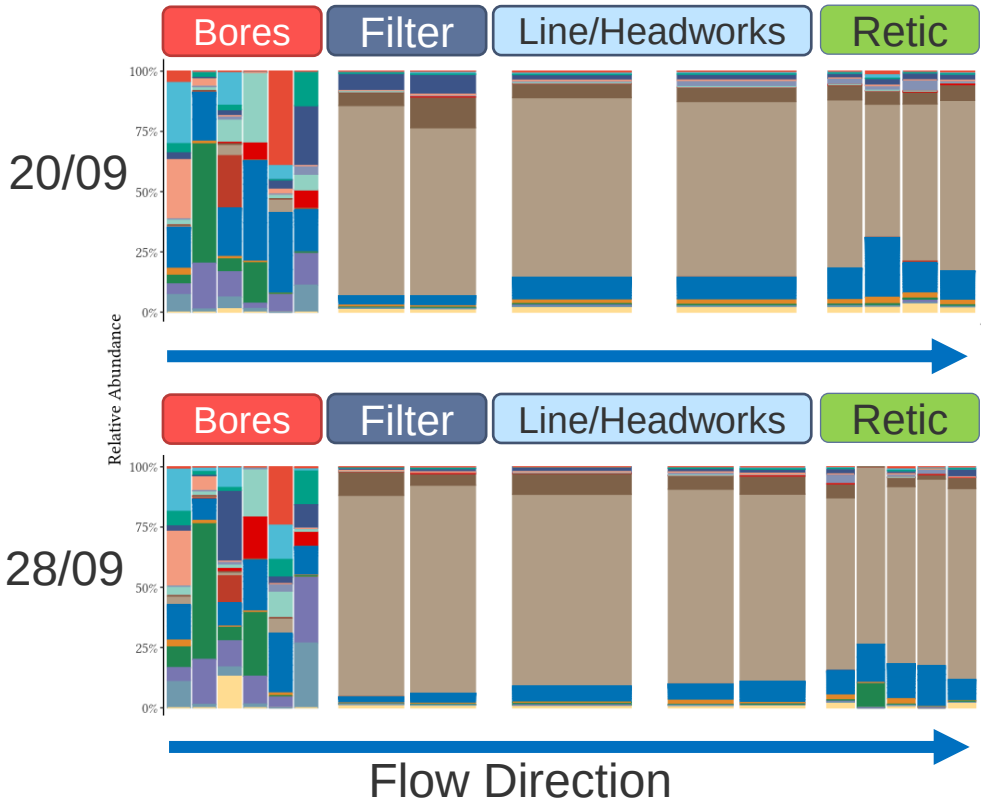


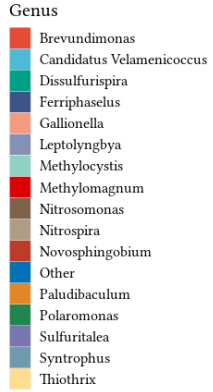
Case Study 3: Woodend

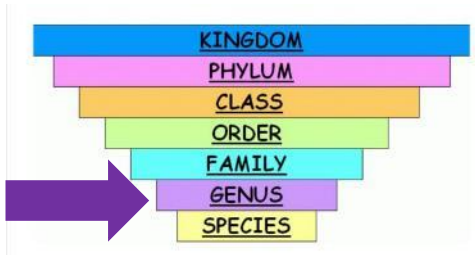
- September 2022, Waimakariri District council
  - Unchlorinated network (at the time)
- Wanted to determine the performance and effect of biological filters.
  - Six bores
    - 140-250m Deep
    - Naturally high Mn Levels
    - Mn MAV <0.4 mg/L
    - Treatment results in <0.0005 mg/L

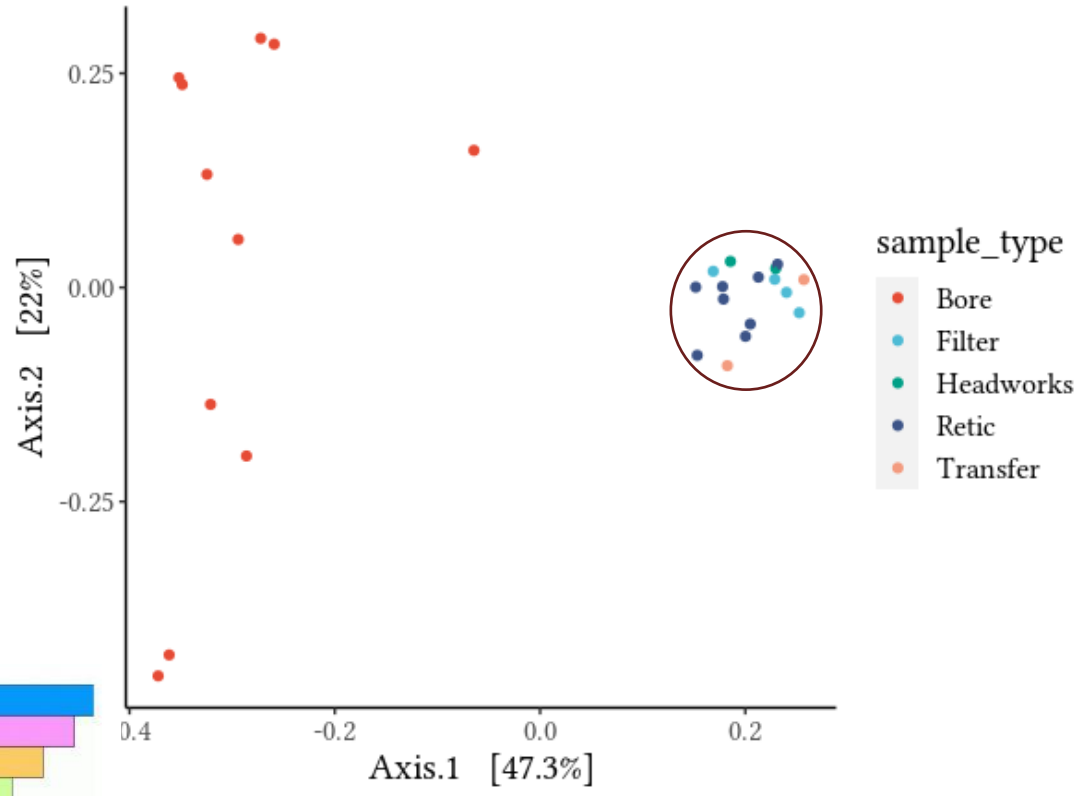


Impact of Manganese Filters









Drinking water Sample Diversity

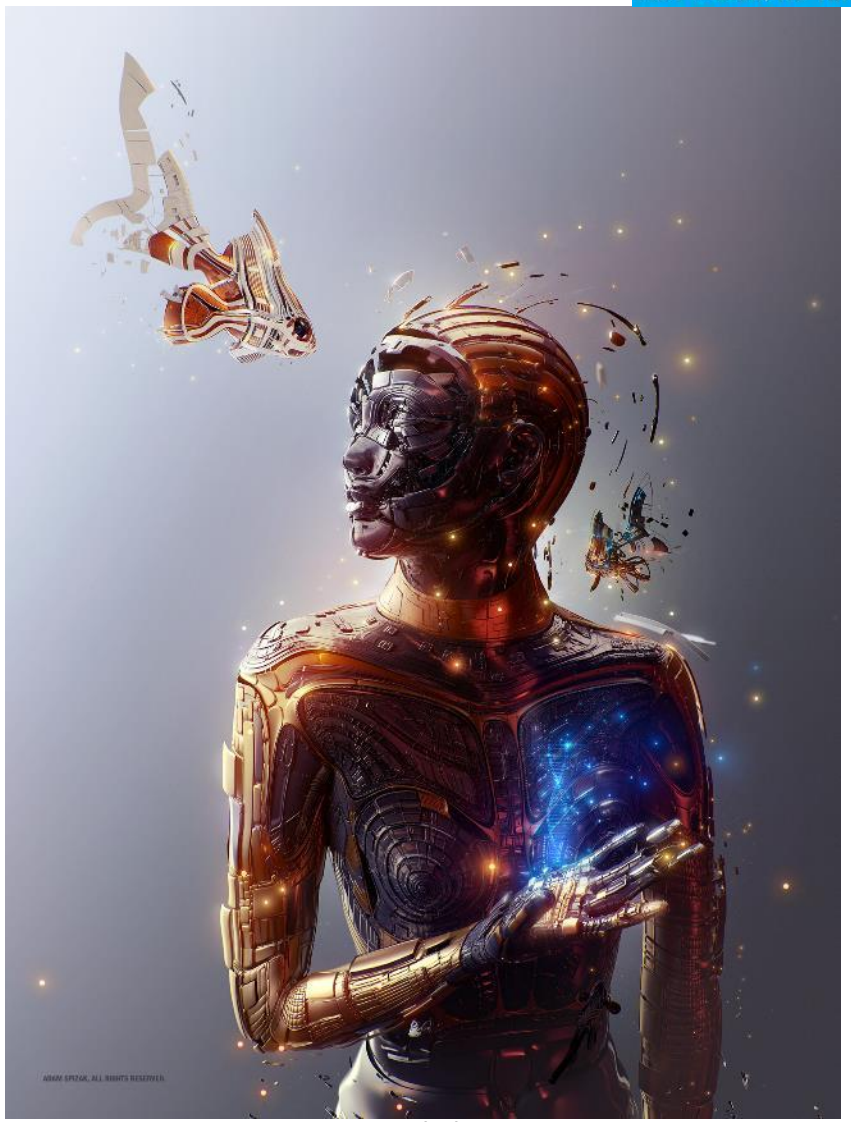
Metagenomic Questions?

What organisms are in a water sample?

- Where are they from?
  - Faecal association?
  - Surface water? Biofilm?
  - Changes over time?
  - Pathogenic organisms?

Where can it be applied?

- Source Water characterisation
- Event/incident investigations
- Network monitoring



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What do we still need to do?

- Quantification
    - Sensitivity and specificity evaluations
- Optimise methodology
    - Database updates/taxonomy changes
    - Integrate technological developments
    - Turnaround and cost

What we need to do it:

- Drinking water samples with total coliforms and/or *E. coli*
- Clear understanding of relationship of samples to each other
- Good metadata - turbidity, chlorination levels, other information

Future plans:

- Viruses
- Protozoa/Amoeba
- Client requests

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Questions? Patai?

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The New Zealand Water & Wastes Association Waiora Aotearoa