HRSD

Cleaning wastewater every day for a better Bay.

Molecular Techniques for Wastewater Analyses

October 4, 2021

Hampton Roads Sanitation District (HRSD)

- HRSD provides wastewater treatment service to 20 cities and counties of southeast Virginia, an area of nearly 5,000 square miles with a population of 1.7 million.
- We operate nine major treatment plants and eight smaller plants in eastern Virginia and the Eastern Shore, with a combined treatment capacity of 249 million gallons per day.

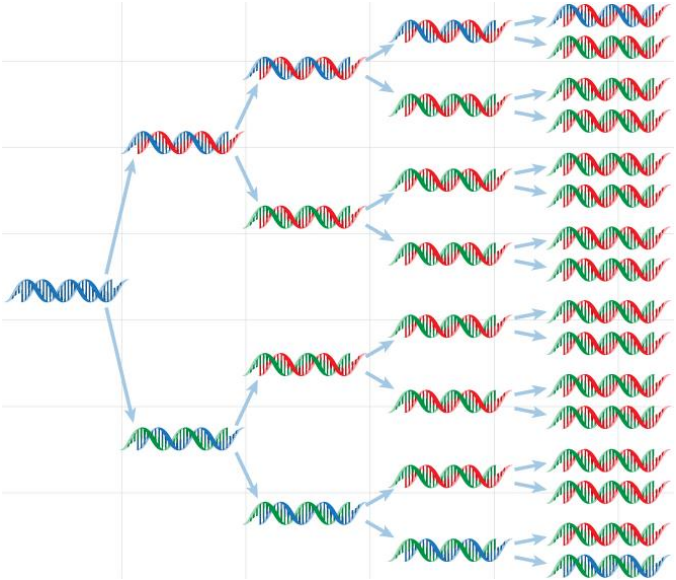
- Mission
  - We protect public health and the waters of Hampton Roads by treating wastewater effectively.
- Vision
  - Future generations will inherit clean waterways and be able to keep them clean.

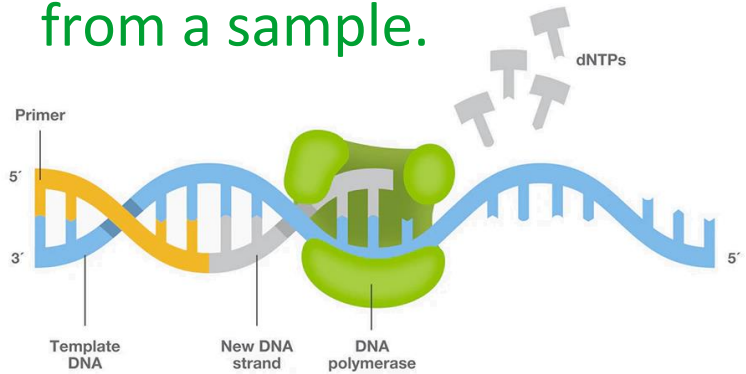
Molecular Technologies

- Molecular biology is a field of science studying cells on their molecular level. It looks at structures such as nucleic acids and proteins which can be used for identification and to provide insight into the function of cells.

  - HRSD uses molecular biology to identify bacteria and viruses that cannot be cultured.
    - PCR testing can be used to identify and quantify a particular DNA fragment, specific to a target of interest.
    - DNA sequencing can be used to characterize the entire bacterial community of a sample.

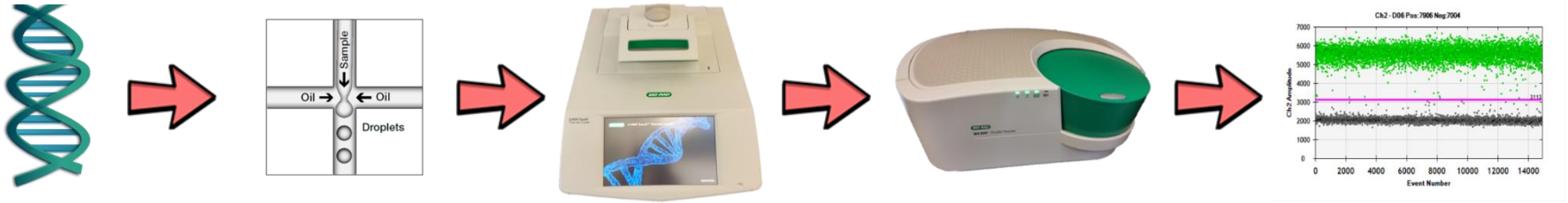
- Polymerase chain reaction (PCR)
  - a method for rapidly replicating DNA to produce billions of copies of a specific DNA fragment. This allows for identification and quantification of a DNA target from a sample.



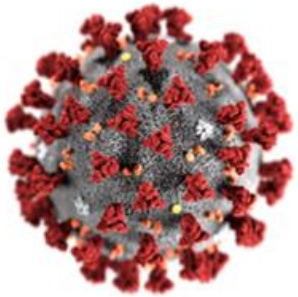


Droplet Digital PCR Workflow

- ddPCR (Droplet Digital PCR)
  - Nucleic acid mixed with enzymes, primers, and fluorescent probe
  - Droplet generation to partition sample
  - Thermal cycling to amplify target sequences
  - Quantification on Droplet Reader



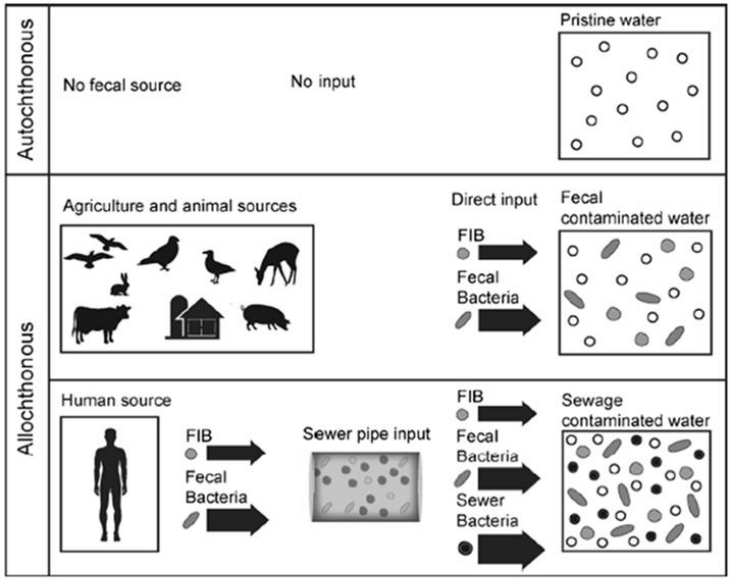
- Microbial Source Tracking
  - Utilizes molecular methods to identify sources of human fecal contamination



- Wastewater-based Epidemiology
  - Uses PCR to track markers in wastewater to make inferences about the population through a pooled community sample.

HRSD

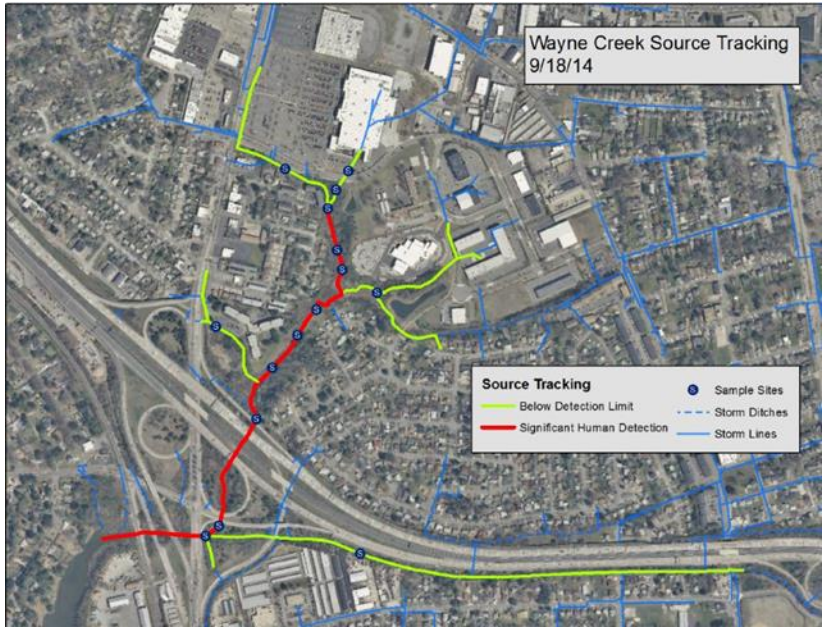
- Microbial Source Tracking (MST) uses ddPCR to detect human-associated fecal markers in contaminated water.
  - Sources of fecal contamination can include wildlife, agriculture, or sewage impairment.
  - HRSD's goal is to determine if contamination is human-related and identify (or at least narrow the search area of) compromised sewer infrastructure so repairs can be made.



Source: O'Mullan et al. 2017

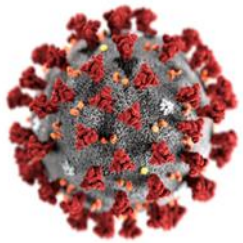
MST: Collection System Investigation

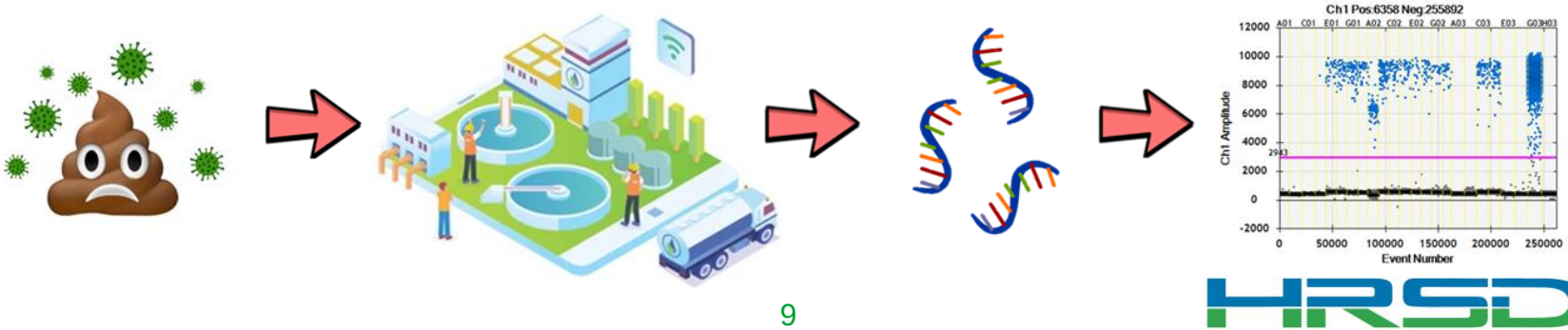
- Identify human contamination
- Follow upstream, sampling at branching points to zoom in on source of contamination



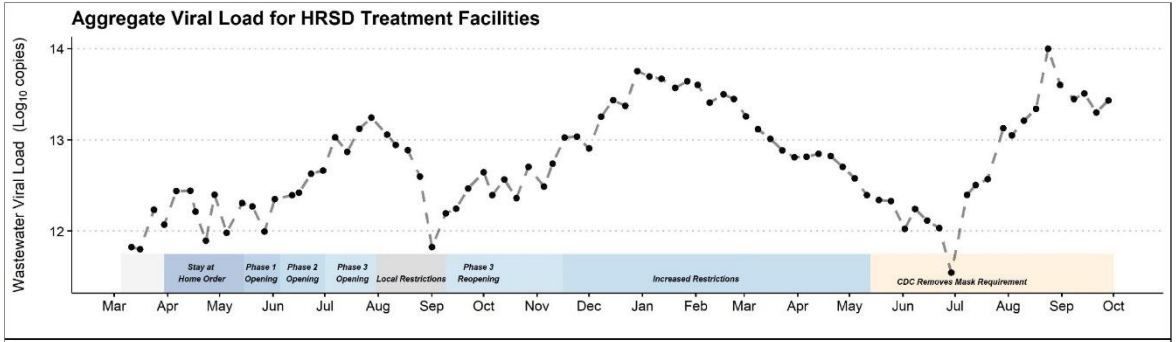
Wastewater-based Epidemiology

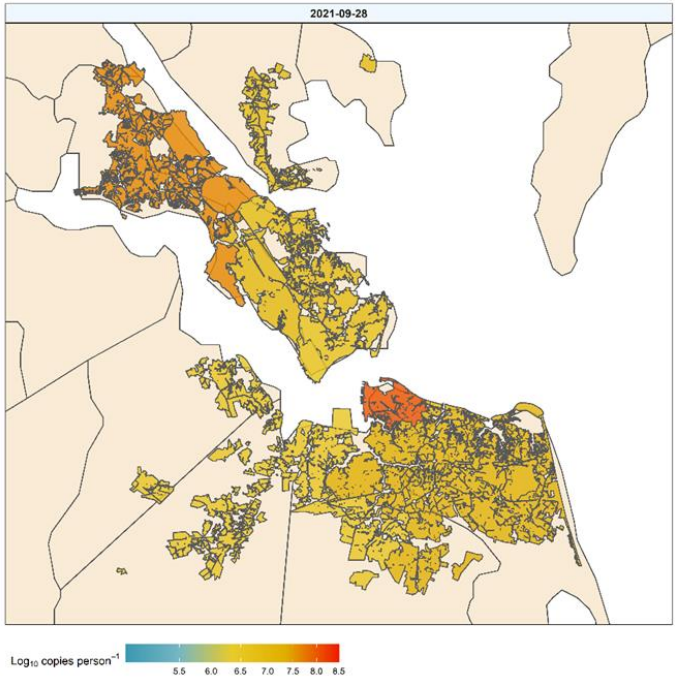
- Wastewater-based epidemiology is a process of looking at various markers in wastewater to make inferences about the population through a pooled community sample.
- SARS-CoV-2 viral particles are shed in feces of infected individuals
- HRSD uses reverse transcriptase PCR (RT-PCR) to detect SARS-CoV-2 in wastewater and determine the regional viral load and where “hotspots” may be occurring





Hampton Roads Regional Viral Load

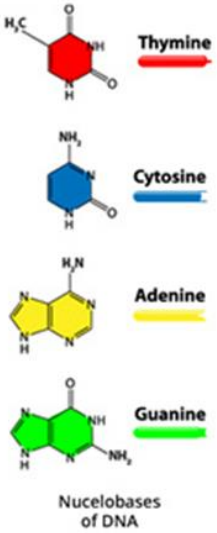




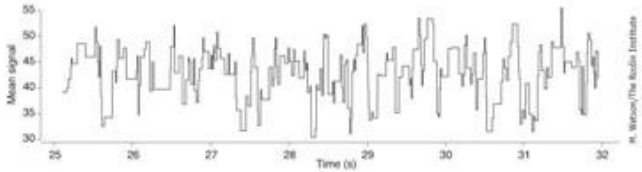
-Bars indicate clinical cases

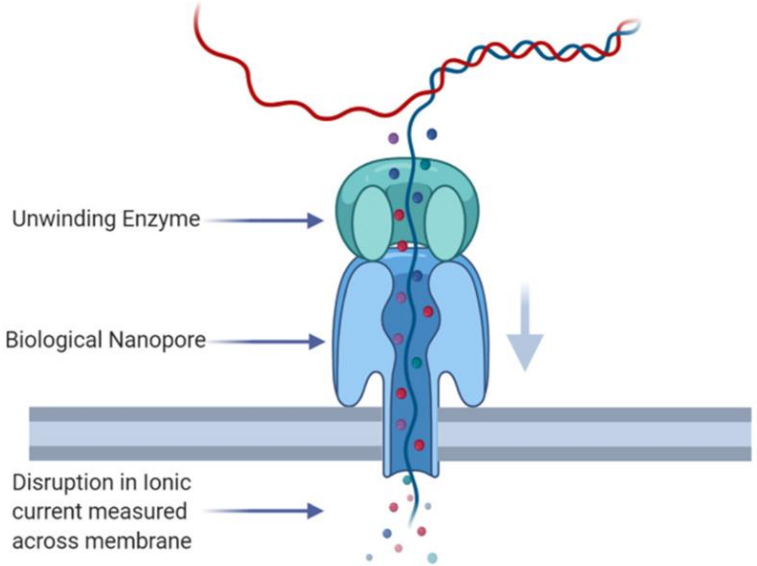
-Points and line indicate SARS-CoV-2 RNA copies in wastewater using a $\log_{10}$ scale

DNA sequencing – determining the order of nucleotide bases in a segment of DNA



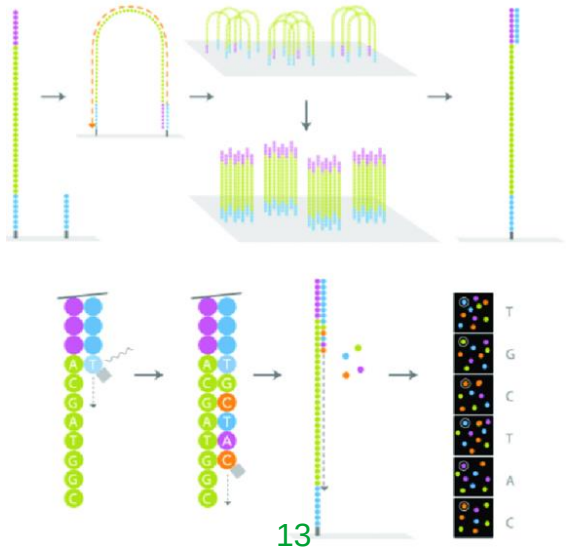
- Nanopore MinION mk1c
  - Long-read sequencing
  - Feeds DNA through a nanopore embedded in a membrane
  - Identifies bases by measuring disruption of electrical current





Source: Lamb et al, 2020

- Illumina iSeq 100
  - Short read sequencing (150x2 bp)
  - Sequencing by synthesis (SBS)



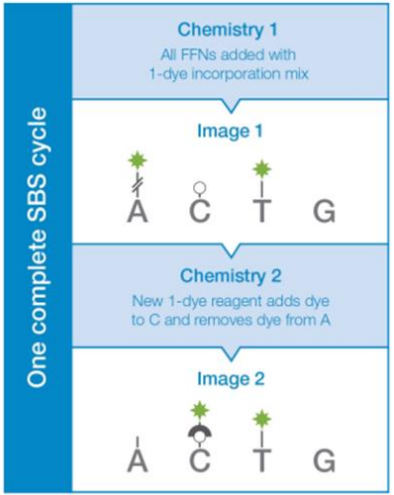
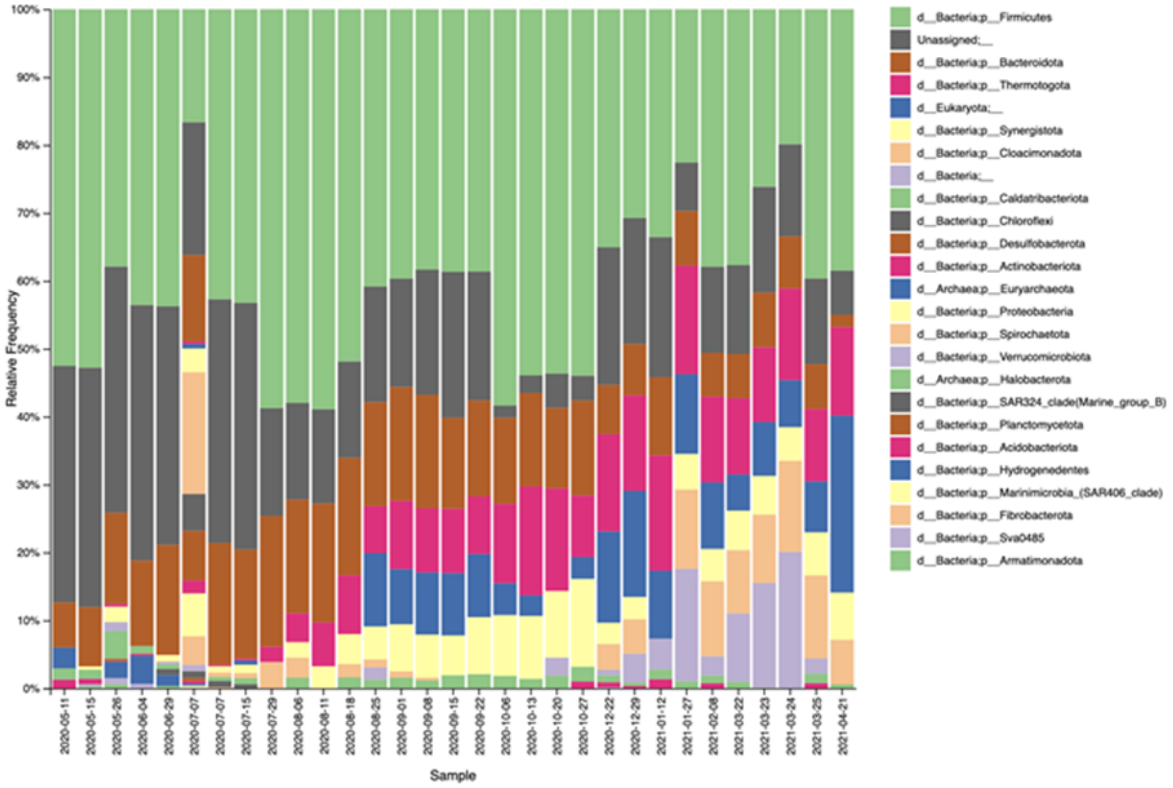


Image 1	Image 2	Result
ON	OFF	A
OFF	ON	C
ON	ON	T
OFF	OFF	G

Sequencing Results

- Sequencing data from treatment plant digestors show change in bacterial community over time.



Contact Information

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  - HRSD Supervising Chemist – Pathogen Section
  - hthompson@hrsd.com

- More information:
  - Gonzalez R, Curtis K, Bivins A, et al. COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. Water Research. Volume 186. 2020. 116296. ISSN 0043-1354. https://doi.org/10.1016/j.watres.2020.116296
  - Gonzalez D, Keeling D, Thompson H, et al. Collection system investigation microbial source tracking (CSI-MST): Applying molecular markers to identify sewer infrastructure failures. Journal of Microbiological Methods. Volume 178. 2020. 106068. ISSN 0167-7012. https://doi.org/10.1016/j.mimet.2020.106068

Questions???