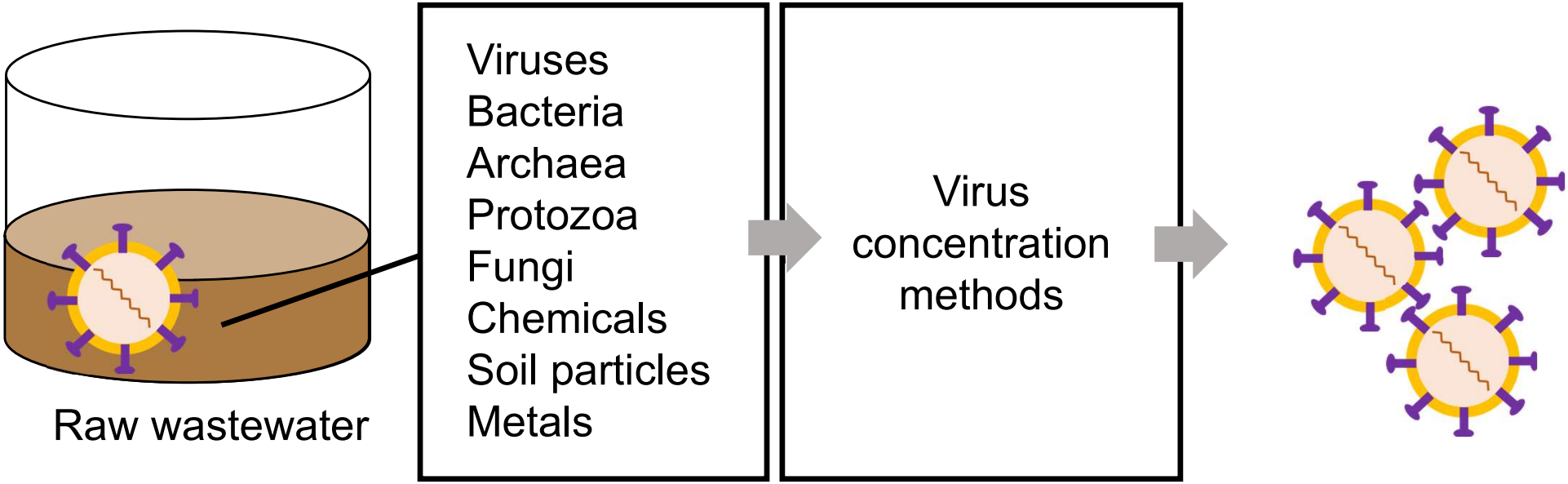
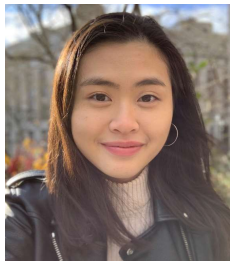
Raw wastewater contains viruses, microbes and chemical substances.



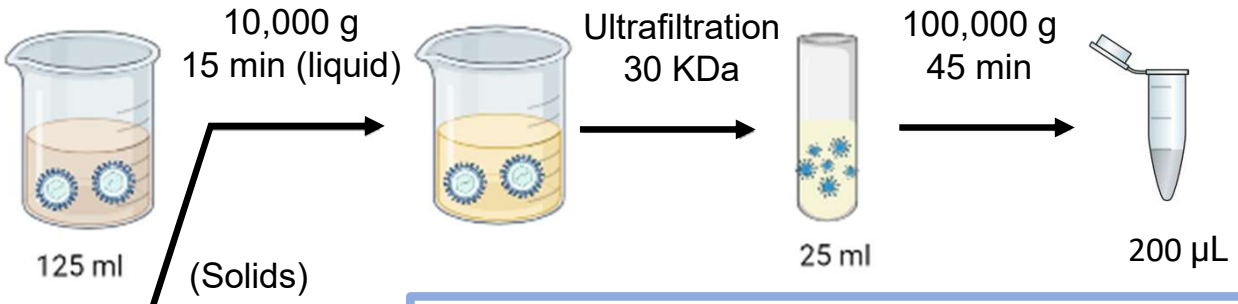
We have trained a team of students to recover wastewater viruses by five different concentration methods.



Vicky Huang

Danya Hanin

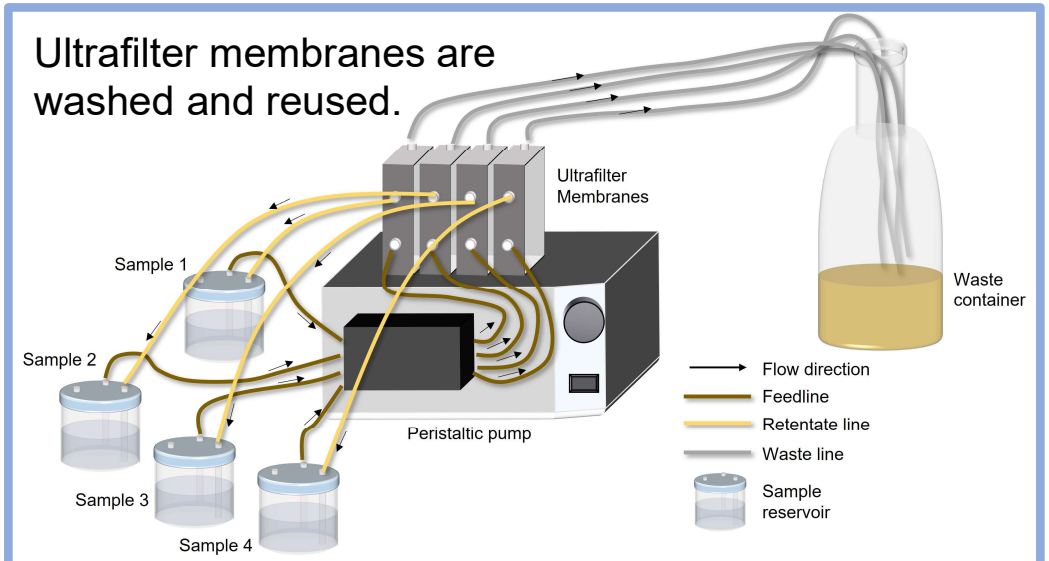
Tangential-flow ultrafiltration (UF)



Solids

~50 mg solids

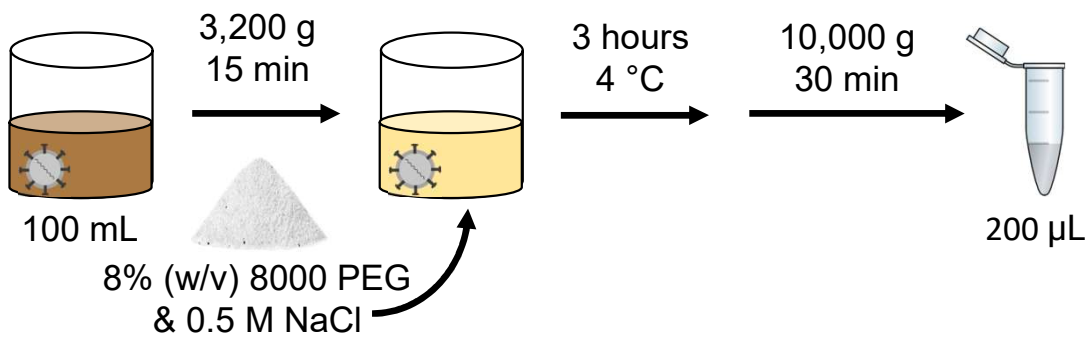
Ultrafilter membranes are washed and reused.



We have trained a team of students to recover wastewater viruses by five different concentration methods.

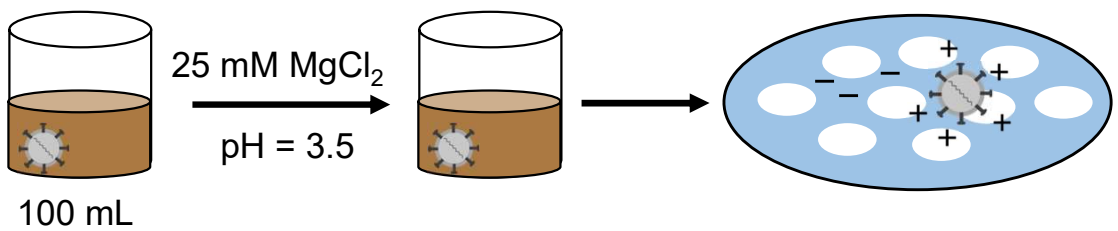
Daniel Bedoya

Polyethylene glycol (PEG) precipitation



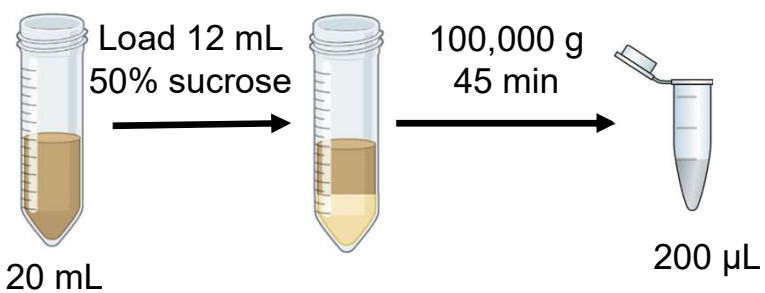
Sydney Gallo

Electronegative membrane filtration (EMF)

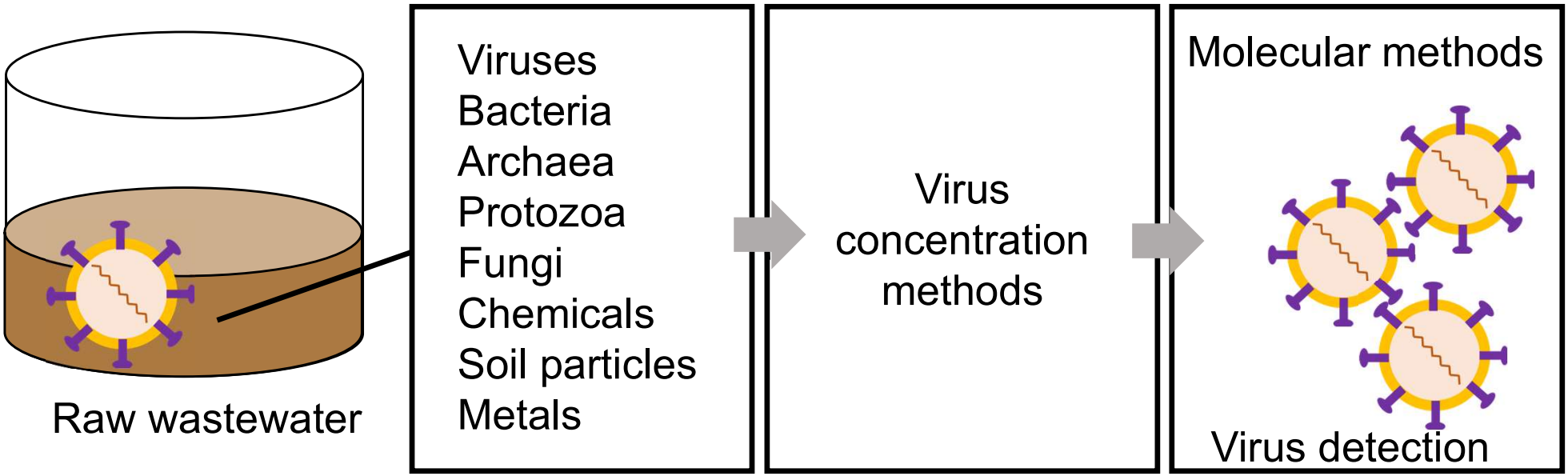


Emily Segelhurst

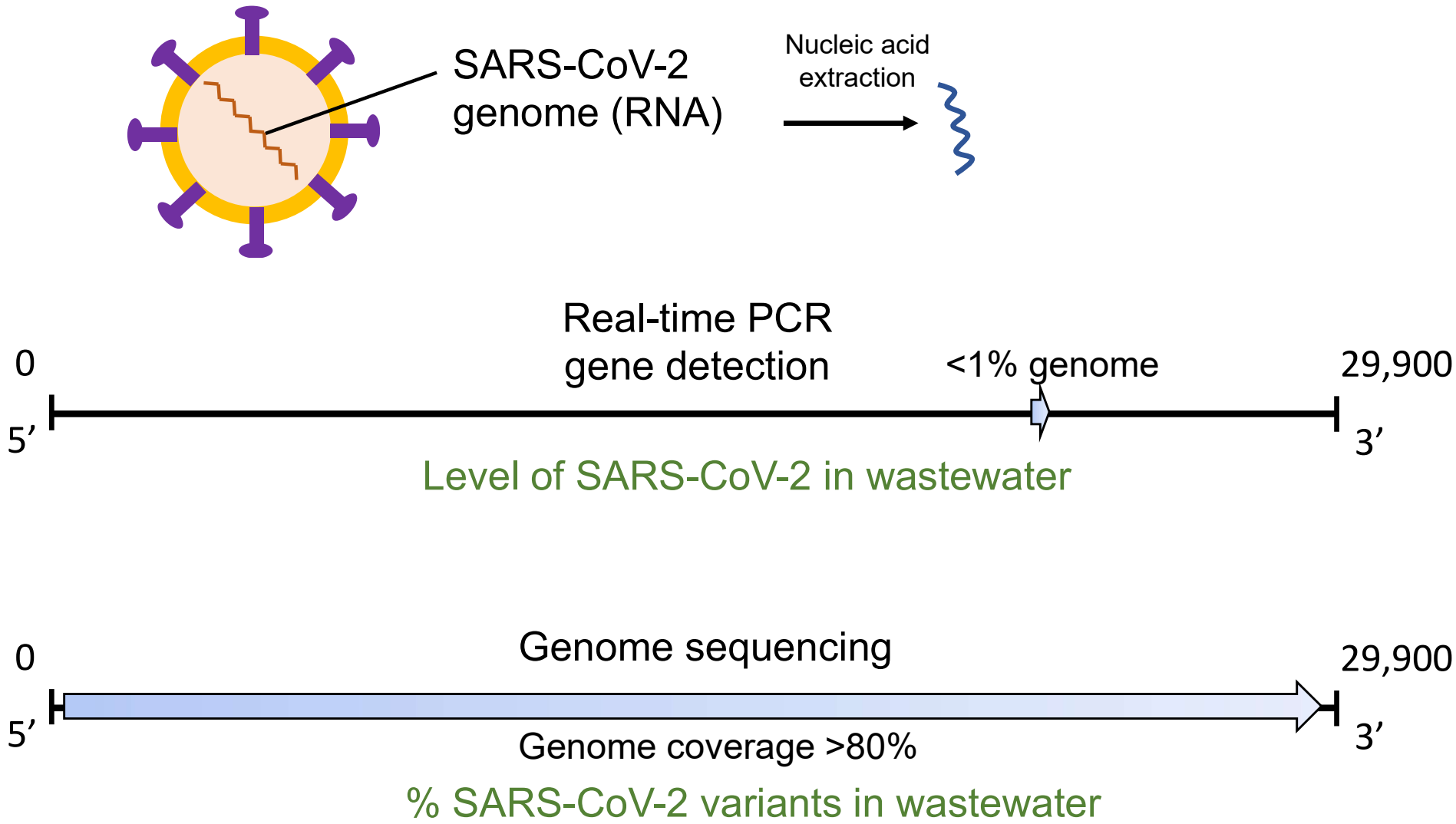
Ultracentrifugation (UG)



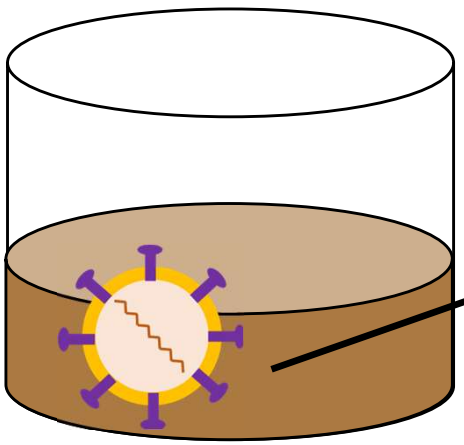
Once the viruses in wastewater are concentrated, downstream analysis is applied for virus detection.



Two molecular methods are used in our lab to detect viruses.



Different virus concentration methods may have various effects on the molecular detection.



Raw wastewater

Viruses
Bacteria
Archaea
Protozoa
Fungi
Chemicals
Soil particles
Metals

Virus concentration methods

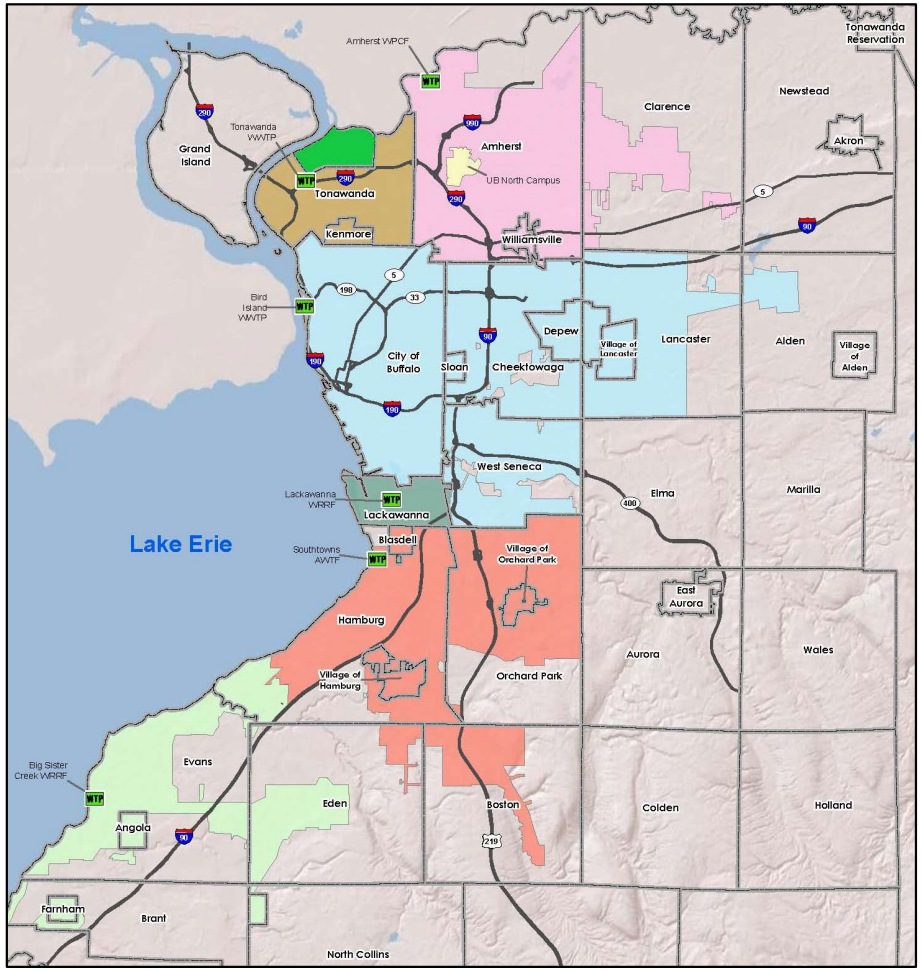
Gene detection

Genome sequencing

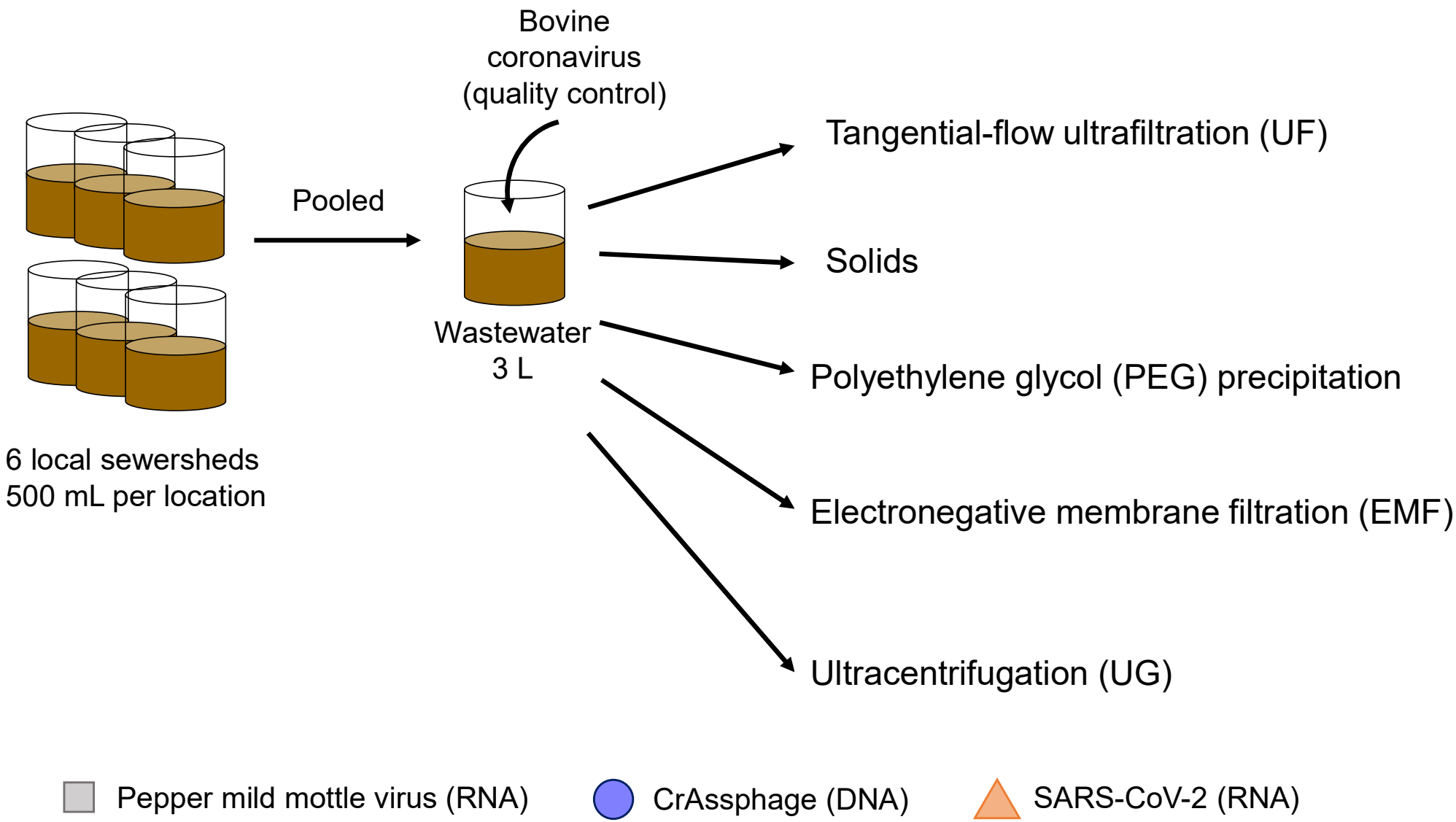
Wastewater samples were collected from local sewersheds.

Sewershed	Permit Flow Rate (MGD)	Population
City of Tonawanda	2.5-5	14,873
Lackawanna	4.5	17,859
Southtowns	16	94,616
Kenmore Tonawanda	25	70,470
Amherst	48	140,324
Bird Island	180	437,357

*MGD: million gallons per day

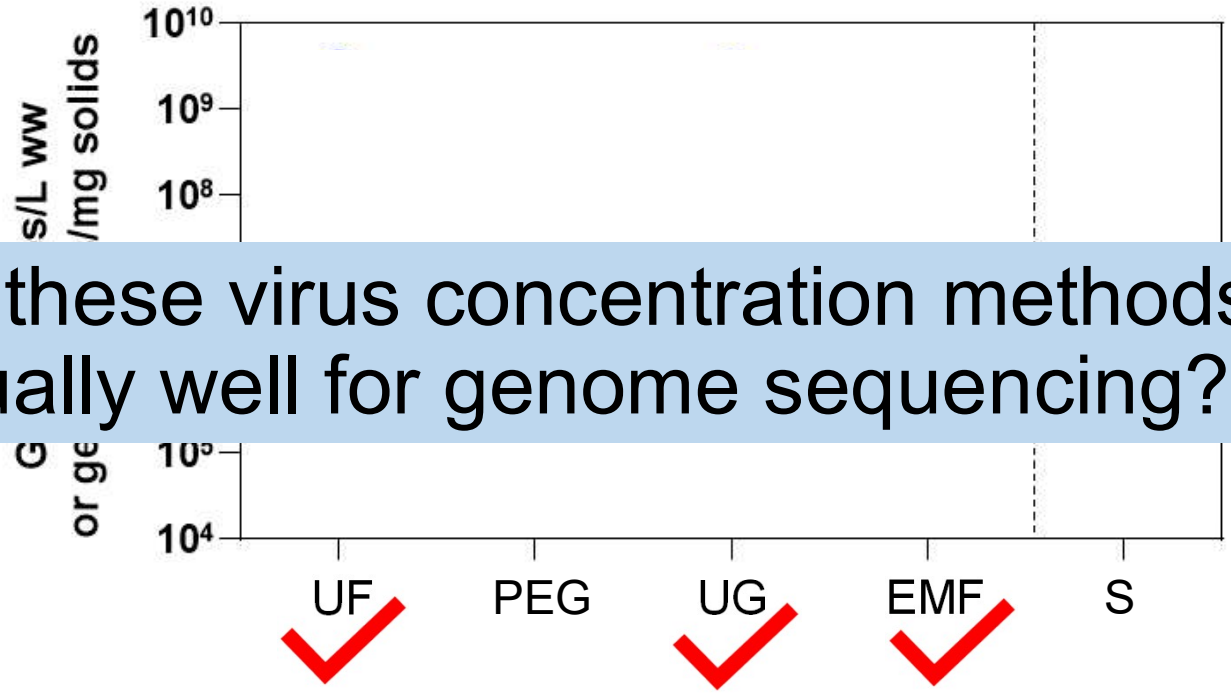


Wastewater samples were pooled, aliquoted, and processed with different virus concentration methods.



Ultrafiltration, membrane filtration, and ultracentrifugation had high recoveries of viral genes from wastewater.

UF: Tangential-flow ultrafiltration
S: Solids
PEG: Polyethylene glycol precipitation
EMF: Electronegative membrane filtration
UG: Ultracentrifugation



Do these virus concentration methods work equally well for genome sequencing?

Pepper mild mottle virus (RNA)
CrAssphage (DNA)
SARS-CoV-2 (RNA)

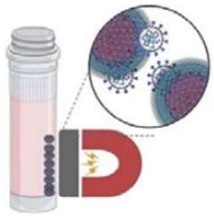
Three virus concentration methods were selected to compare their performance on genome sequencing.

Concentration method	Virus selectivity	
Tangential-flow filtration (UF)	Viruses in the liquid (solids removed by 10,000 g centrifugation)	Nonselective
Electronegative membrane filtration (EMF)	Viruses co-concentrated with other microbes	Nonselective

Joyce Wang

10 mL

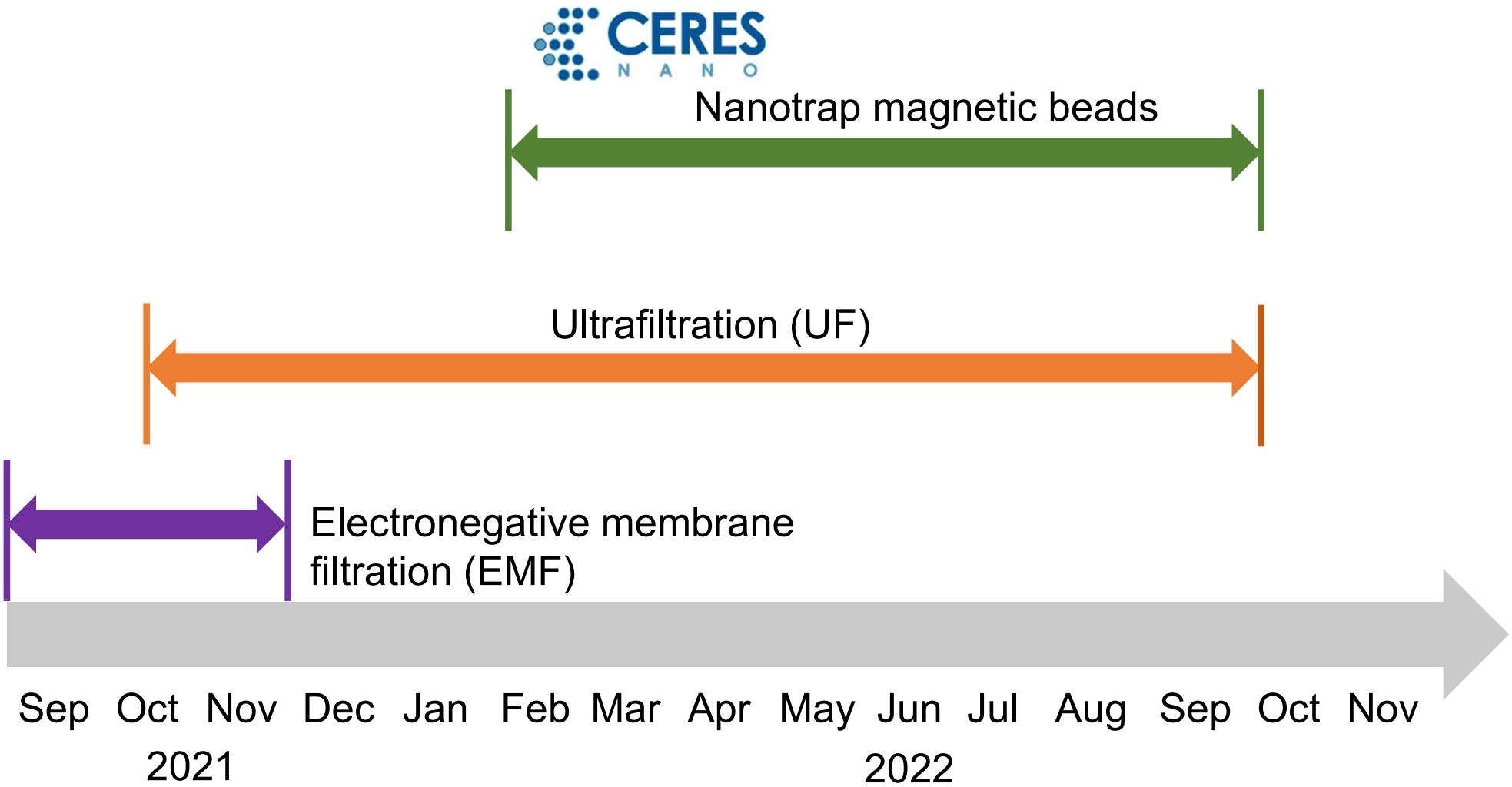
Mix with Nanotrap Microbiome A Particles



Nucleic acid extraction

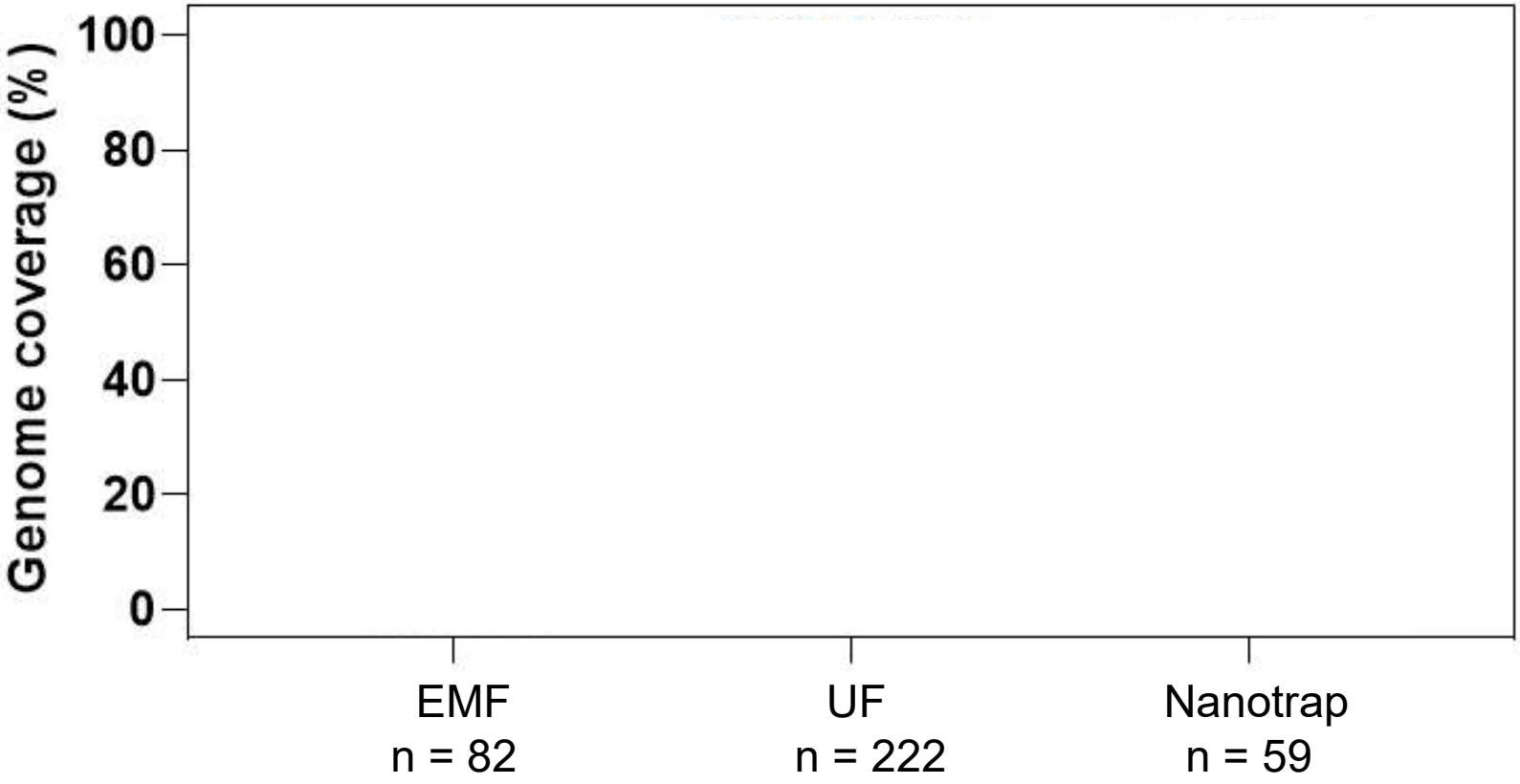
CERES NANO

Three virus concentration methods were selected to compare their performance on genome sequencing.

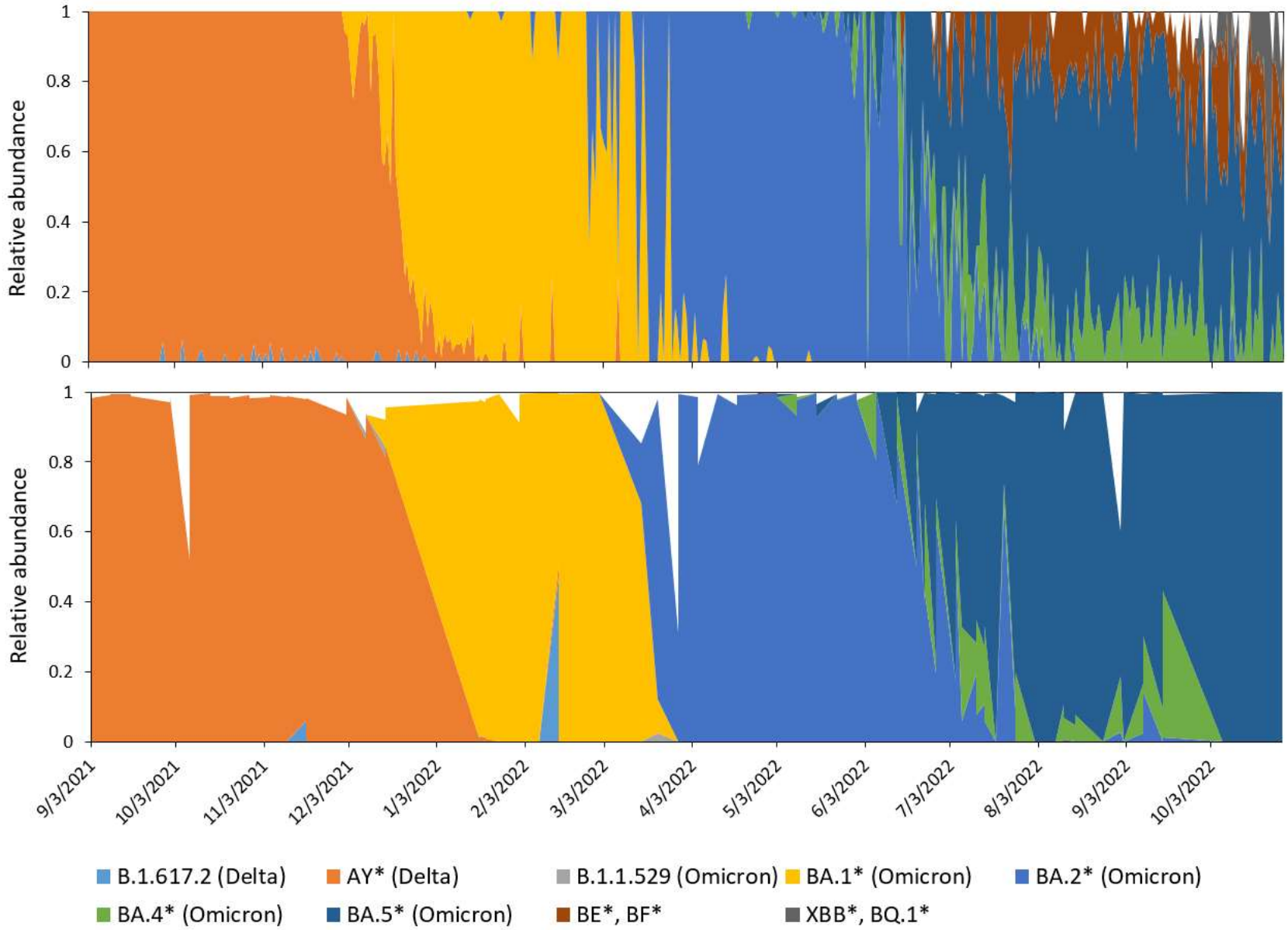


Tangential-flow ultrafiltration (UF) and Nanotrap yielded higher coverage of SARS-CoV-2 genome.

UF: Tangential-flow ultrafiltration
EMF: Electronegative membrane filtration
Nanotrap: Nanotrap magnetic beads



The relative abundances of SARS-CoV-2 variants are comparable between clinical and wastewater data.

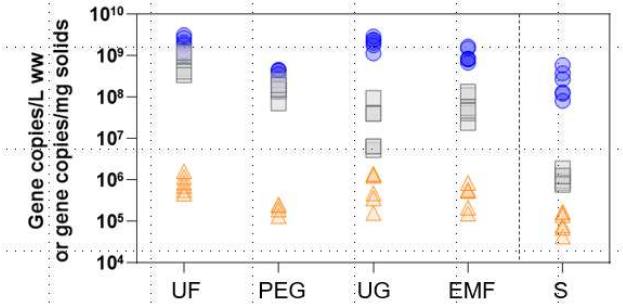


Clinical data
Erie County

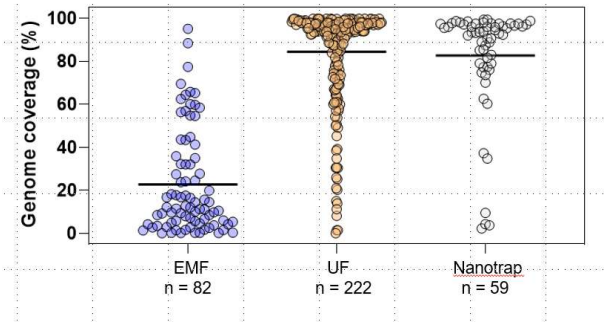
Wastewater data
Erie County

Conclusions

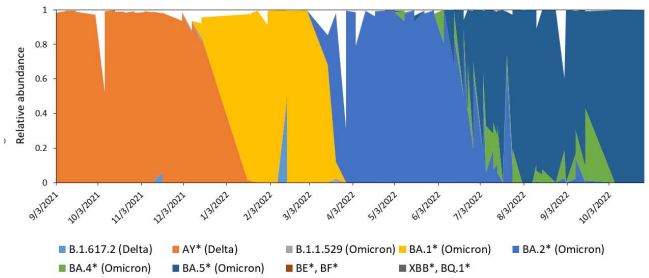
- Among the nonselective virus concentration methods, tangential-flow ultrafiltration, electronegative membrane filtration, and ultracentrifugation have high recoveries of viral genes from wastewater.



- Tangential-flow ultrafiltration and Nanotrap magnetic beads recover viral genomes from wastewater for reliable performance of SARS-CoV-2 genome sequencing.



- Wastewater genome sequencing of SARS-CoV-2 is an effective approach to track various SARS-CoV-2 variants circulating in the community, but bioinformatic tools and sequencing method need to be updated.



Future work

- Develop workflow to monitor other viruses of concern, such as respiratory viruses (respiratory syncytial virus, influenza virus) and enteric viruses (hepatitis A virus, norovirus).

- Integrate wastewater data with other databases (transportation, vaccination rates, social media, community engagement) to develop an early warning system for future pandemics.

Acknowledgements

UB Genomics and Bioinformatics Core: Jennifer Surtees, Don Yergeau, Jonathan Bard, Natalie Lamb, Alyssa Pohlman, Amanda Boccolucci, Jamaal Emerson, Brandon Marzullo, Norma Nowak

UB Wastewater Committee: John Tomaszewski, Jean Wactawski-Wende, Tom Russo, Jeff Angiel, Brian Foti, Greg Wilding, Craig Abbey, Brian Haggerty

New York State Wastewater Network: Milagros Neyra, Dustin T. Hill, Lydia J. Bennett, Christopher Dunham, Mohammed Alazawi, Brenden Bedard, Mary Collins, Austin Fehrman, Christopher Gobler, Alejandro Godinez, Hyatt Green, Brianna Hanson, Tabassum Insaf, Haley Kappus-Kron, Daniel Lang, Elliot J. Moran, Dana Neigel, Shailla J. Raymond, David A. Larsen

Erie County collaborators: Joseph Fiegl, Gale Burstein