

Using Genomic Tools to Interrogate Infectious Diseases and Genetic Disorders in Southern Nevada

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Chang, 2020



Laboratory of Neurogenetics and Precision Medicine





Collaborators



RESEARCH, INNOVATION & IMPACT
Water & Energy Sustainable
Technology Center



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Funding



National Institute of
General Medical Sciences



National Institute
of Mental Health



National Institute of Dental
and Craniofacial Research



BRAIN &
BEHAVIOR
RESEARCH FOUNDATION

Awarding **NARSAD** Grants



NIH grants: GM121325, GM103440, DE029954, and MH109706. CDC grant: NH75OT000057-01-00. CARES Act grant from the Nevada Governor's Office of Economic Development.

A role for cilia in development and disease

Anosmia

Cognitive defects

Retinal degeneration

Hearing loss

Craniofacial abnormalities

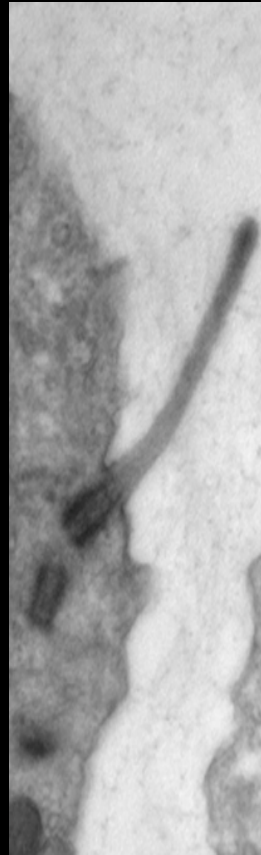
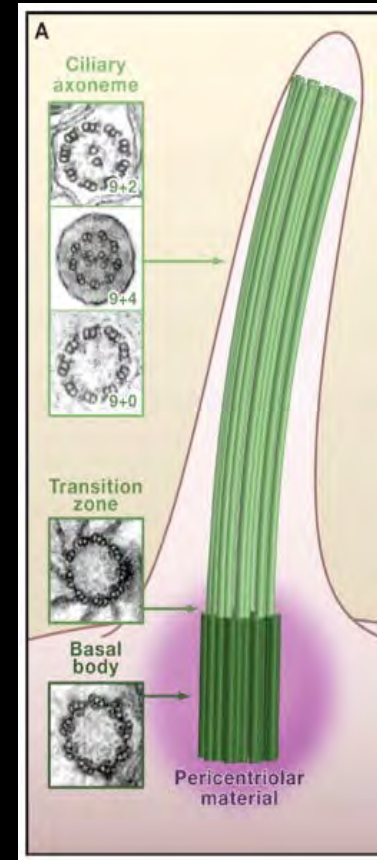
Respiratory defects

Polydactyly

Renal and pancreatic cysts

Sterility

Obesity



Oh et al., Cell Metabolism 2015

Gerdes et al., Cell 2009

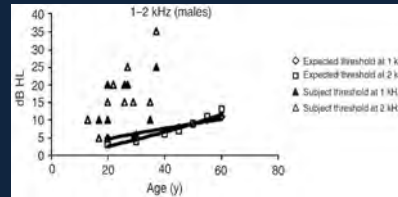
Defects of cilium have been implicated in a number of human diseases



Skeletal abnormalities



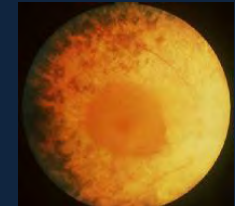
*Central/
peripheral
nervous
system defects*



Hearing loss



*Renal,
pancreatic,
biliary
cysts/fibrosis*



*Retinal
degeneration*

Genomic tools to discover mutations that cause disease

Nephronophthisis
(NPHP)

Oral-Facial-Digital Syndrome
(OFD)

Meckel-Gruber Syndrome
(MKS)

MILD

SEVERE

Senior-Loken Syndrome
(SLS)

Bardet-Biedl Syndrome
(BBS)

Jeune Asphyxiating
Thoracic Dystrophy
(JATD)

Ciliopathies: An expanding group of overlapping clinical entities

Learning objectives

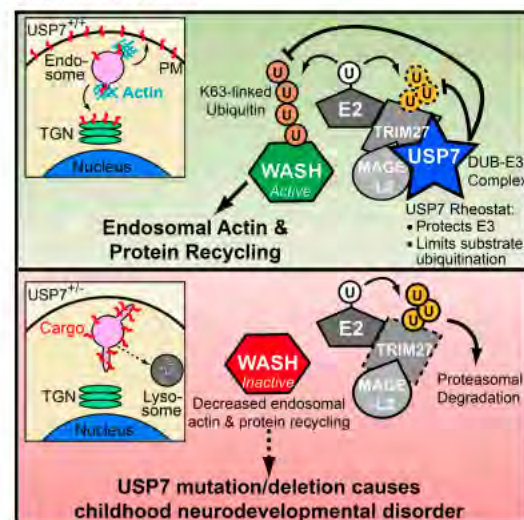
- **Objective 1:** Demonstrate how copy number variation can be resolved using functional genomics
- **Objective 2:** Define how sequencing technologies can be used to interrogate COVID-19 using clinical and wastewater samples

Key findings

Molecular Cell

USP7 Acts as a Molecular Rheostat to Promote WASH-Dependent Endosomal Protein Recycling and Is Mutated in a Human Neurodevelopmental Disorder

Graphical Abstract



Authors

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Correspondence

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In Brief

Hao et al. describe a function of the USP7 deubiquitinating enzyme in regulation of WASH/retrorner-mediated endosomal protein recycling. USP7 functions as a molecular rheostat to prevent auto-ubiquitination and proteasomal degradation of TRIM27 E3 ubiquitin ligase, but also deubiquitinates WASH. Genetic studies identify cases of USP7 mutation/deletion resulting in a human neurodevelopmental disorder that overlaps with MAGEL2 mutation.

Article

ARTICLE | Genetics
in Medicine

Open

Pathogenic variants in *USP7* cause a neurodevelopmental disorder with speech delays, altered behavior, and neurologic anomalies

A full list of authors and affiliations appears at the end of the paper.

Purpose: Haploinsufficiency of *USP7*, located at chromosome 16p13.2, has recently been reported in seven individuals with neurodevelopmental phenotypes, including developmental delay/intellectual disability (DD/ID), autism spectrum disorder (ASD), seizures, and hypogonadism. Further, *USP7* was identified to critically incorporate into the MAGEL2-USP7-TRIM27 (MUST), such that pathogenic variants in *USP7* lead to altered endosomal F-actin polymerization and dysregulated protein recycling.

Methods: We report 16 newly identified individuals with heterozygous *USP7* variants, identified by genome or exome sequencing or by chromosome microarray analysis. Clinical features were evaluated by review of medical records. Additional clinical information was obtained on the seven previously reported individuals to fully elucidate the phenotypic expression associated with *USP7* haploinsufficiency.

Results: The clinical manifestations of these 23 individuals suggest a syndrome characterized by DD/ID, hypotonia, eye anomalies,

feeding difficulties, GERD, behavioral anomalies, and ASD, and more specific phenotypes of speech delays including a nonverbal phenotype and abnormal brain magnetic resonance image findings, including white matter changes based on neuroradiologic examination.

Conclusion: The consistency of clinical features among all individuals presented regardless of de novo *USP7* variant type supports haploinsufficiency as a mechanism for pathogenesis and refines the clinical impact faced by affected individuals and caregivers.

Genetics in Medicine (2019) 21:1797–1807; <https://doi.org/10.1038/s41436-019-0433-1>

Keywords: *USP7*; neurodevelopment; speech delay; white matter paucity; corpus callosum thinning

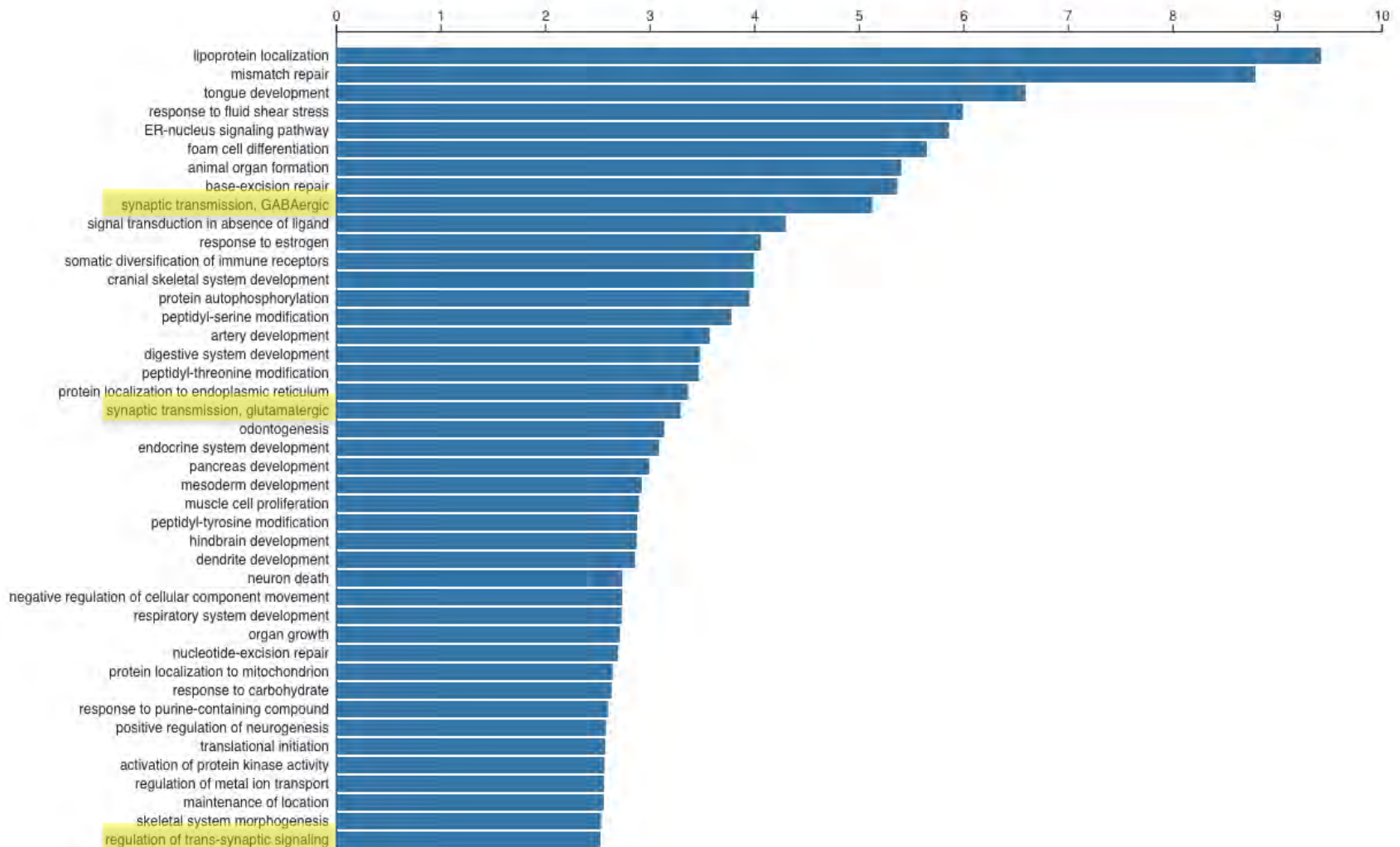
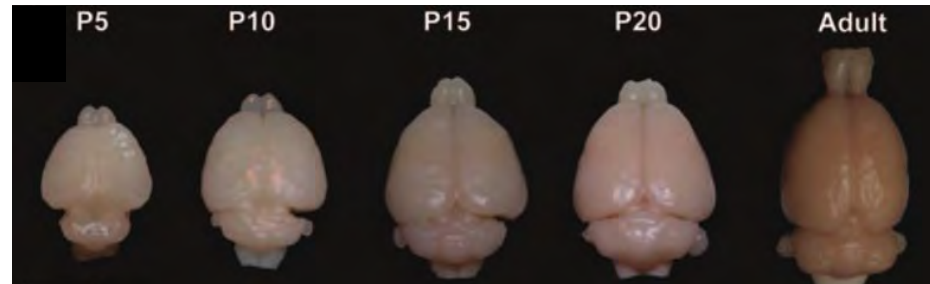
Two individuals with duplication at the 16p13.2 locus

- GI: vomiting, GERD, feeding difficulties with weight loss, failure to thrive
- Autism and sleep disturbances
- Seizures and speech difficulties
- **Array: arr[hg19] 16p13.2x3 (4 or 5 genes)**

Questions

- What does USP7 bind to in the brain?
- What proteins/substrates are regulated in the brain when there is:
 - 1) Too little USP7
 - 2) USP7 with a variant
 - 3) Too much USP7
- What pathways are regulated by USP7?
- How do excitatory neurons behave/fire when you titrate USP7 levels?

USP7 interactome in the brain



Interactors vs. substrates?

Conditions - embryonic hippocampal neurons exposed to:

- 1) Chemical inhibition – P5091 and FT671
- 2) Genetic inhibition – shRNA USP7
- 3) Human patient-induced NPCs
- 4) Overexpression of human USP7

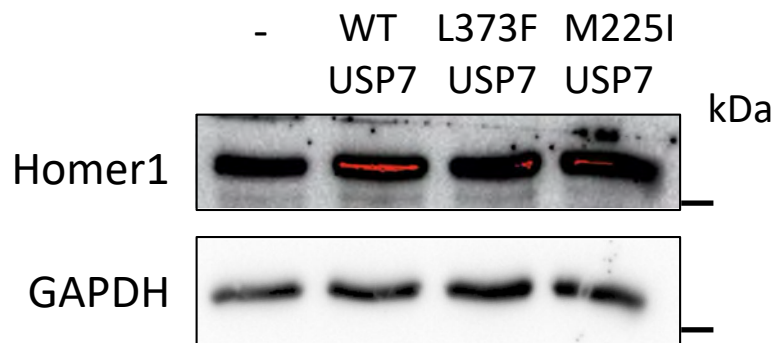
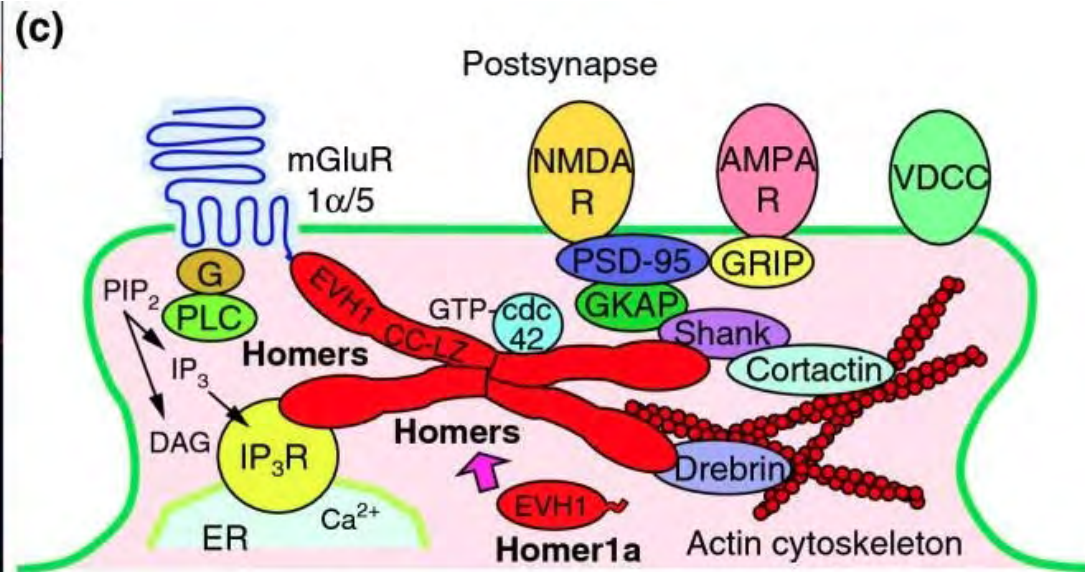
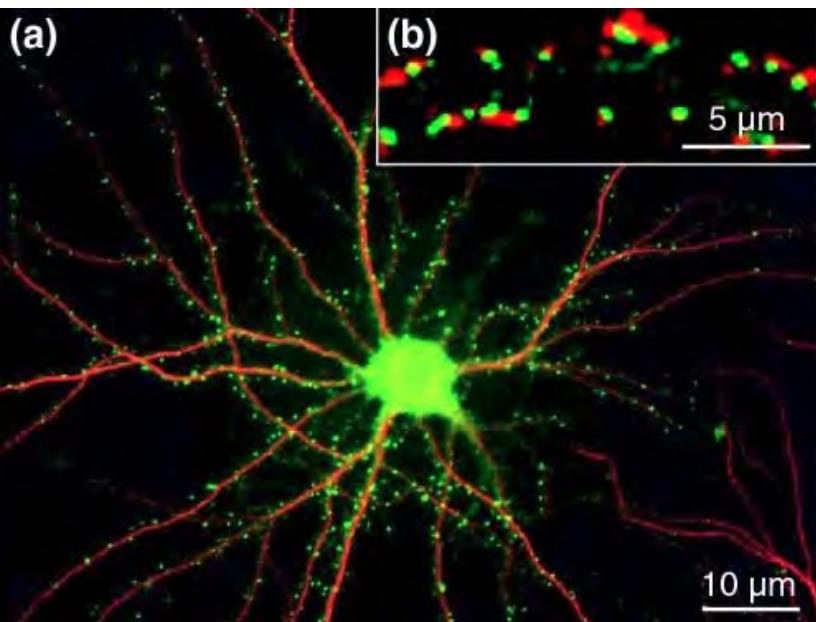
**** Synaptic signaling**

**** Aminoacyl-tRNA synthetase function**

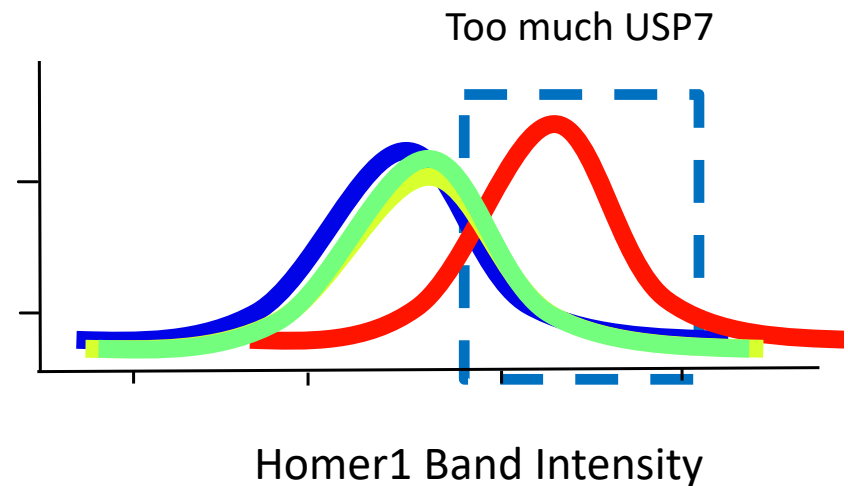
Hypothesis

USP7 is required to stabilize proteins at the synapse which is necessary for excitatory and inhibitory signaling

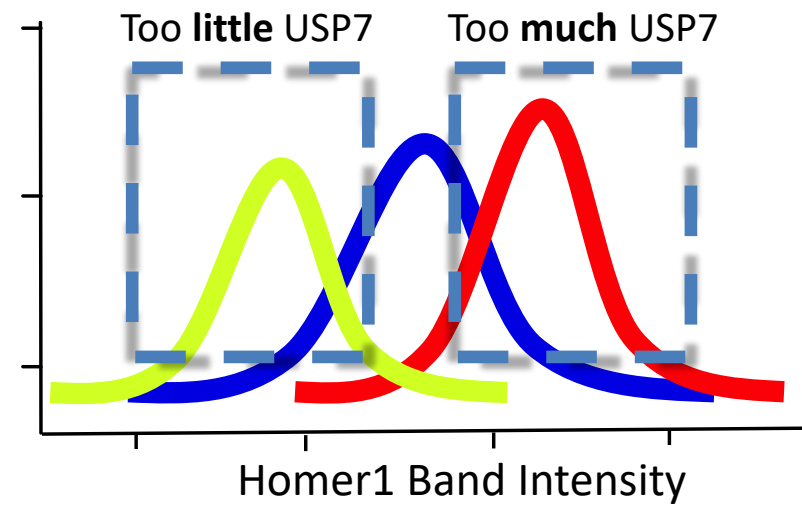
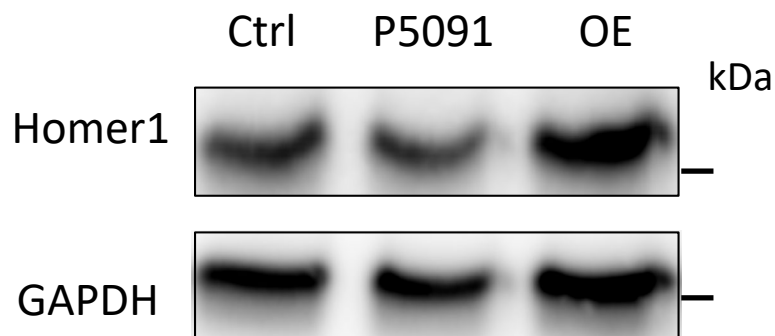
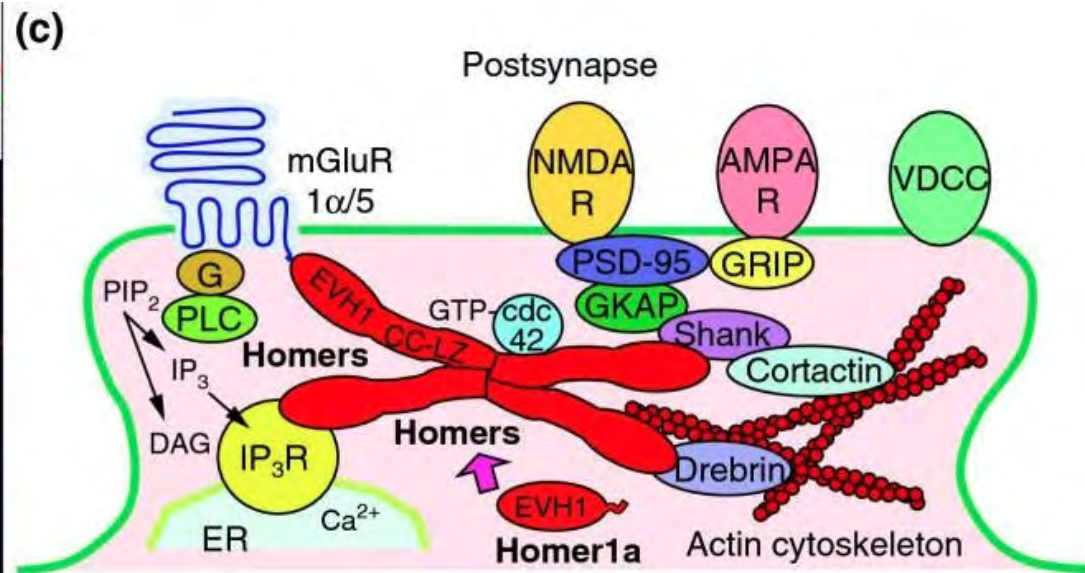
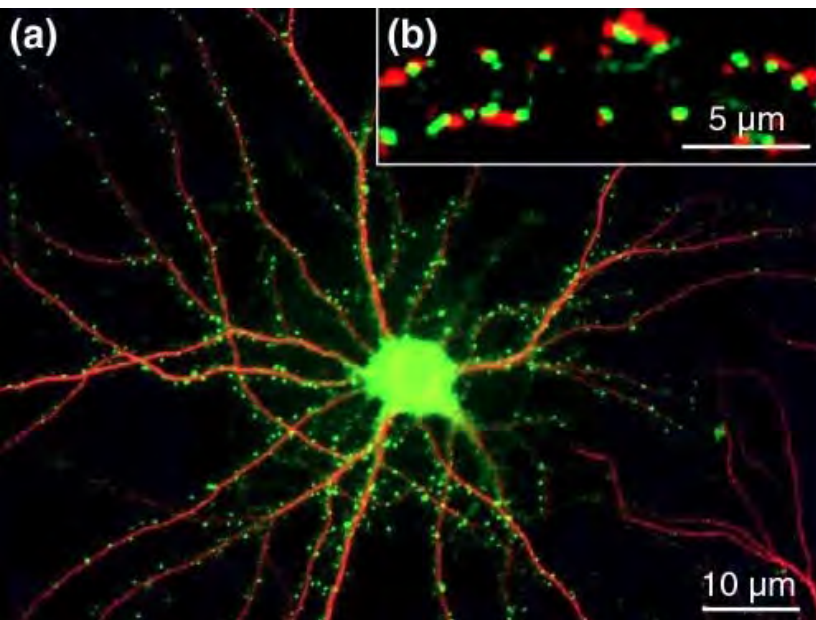
Substrates



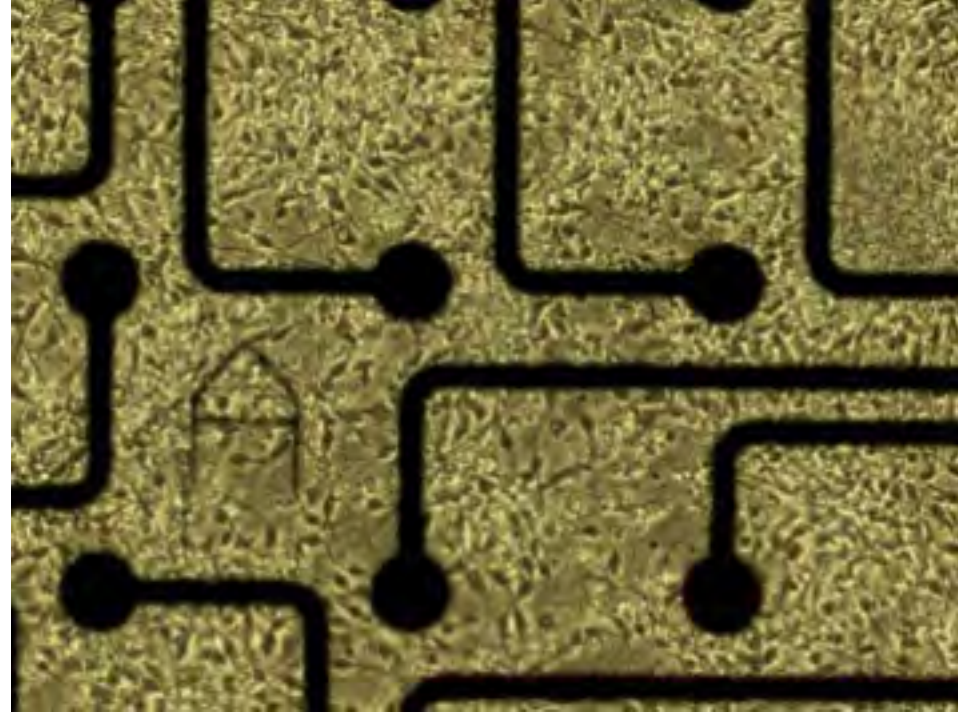
Similar results for IPSC-induced L373F neurons



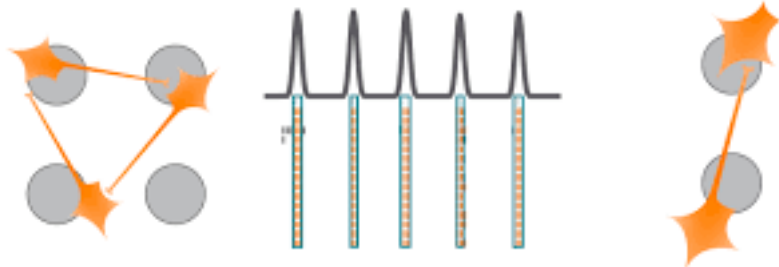
Substrates



Neuronal activity upon changing USP7 levels

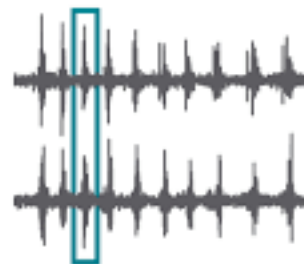


Bursts of Action Potentials



Oscillation

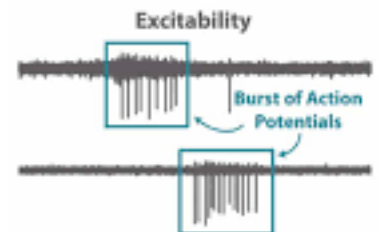
Connectivity



Synchrony

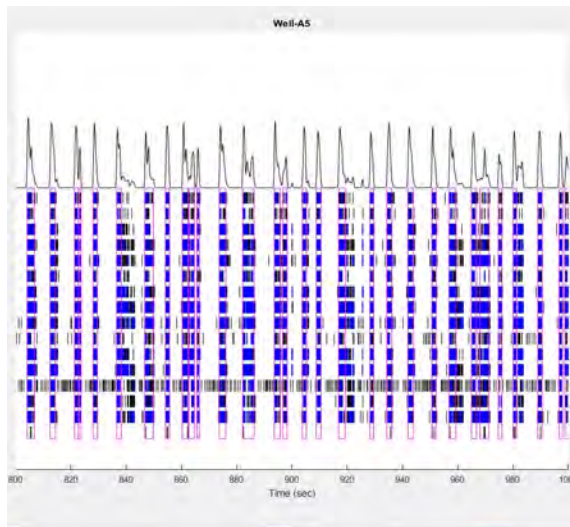


Excitability

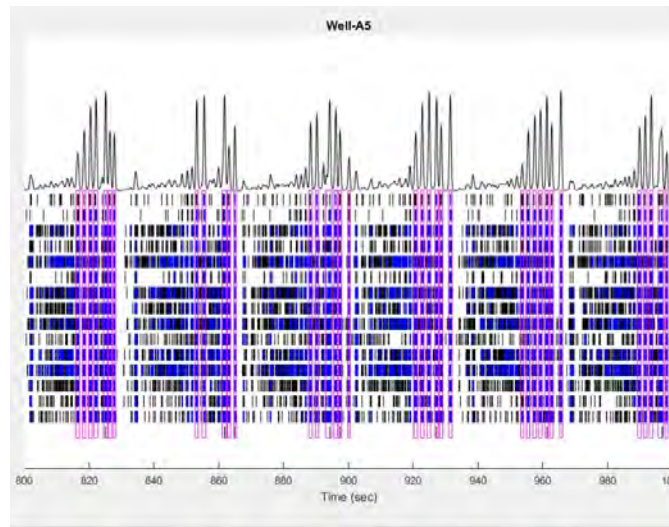


Phenotypes

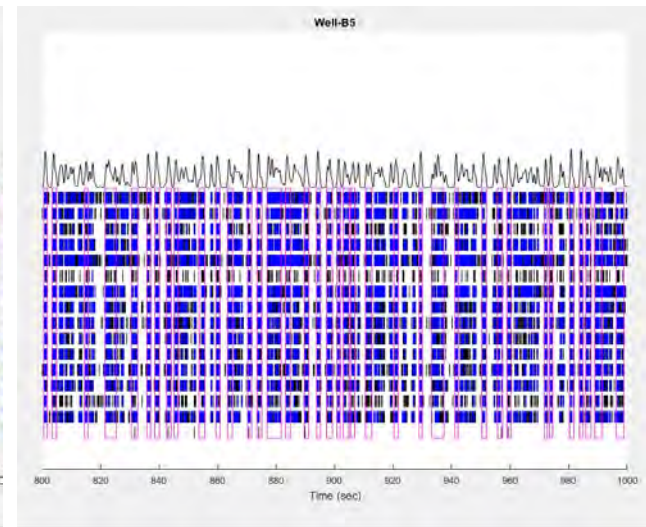
Control



Inhibition



Overexpression



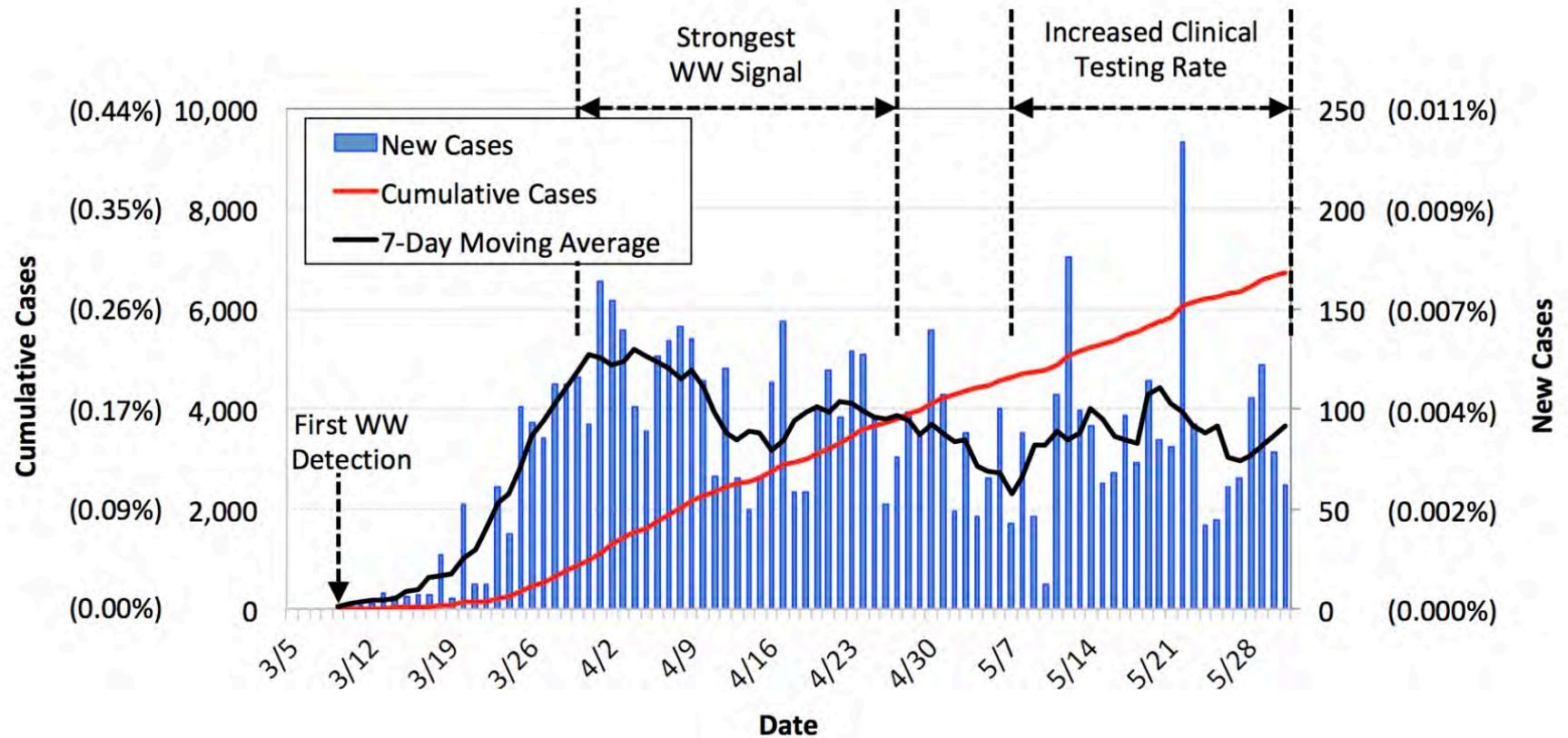
Conclusions and ongoing experiments

- Regulation of USP7 levels is critical – too much and too little may give similar phenotypes (this is **KEY** for gene therapy approach)
- Common theme of synaptic signaling changes and protein translation defects observed
- USP7 localization at the synapse during development (and homeostasis) necessary for scaffolding of synaptic receptors
- Repeat our conditions with USP variants
- Develop drug screen with human iPSC-derived neurons to examine rescue of phenotypes
- Identify new patients and characterize clinical symptoms

April 2020

- Can we use genomic tools to track SARS-CoV-2?

Detection of SARS-CoV-2 in wastewater



SNWA: Dan Gerrity and Katerina Papp

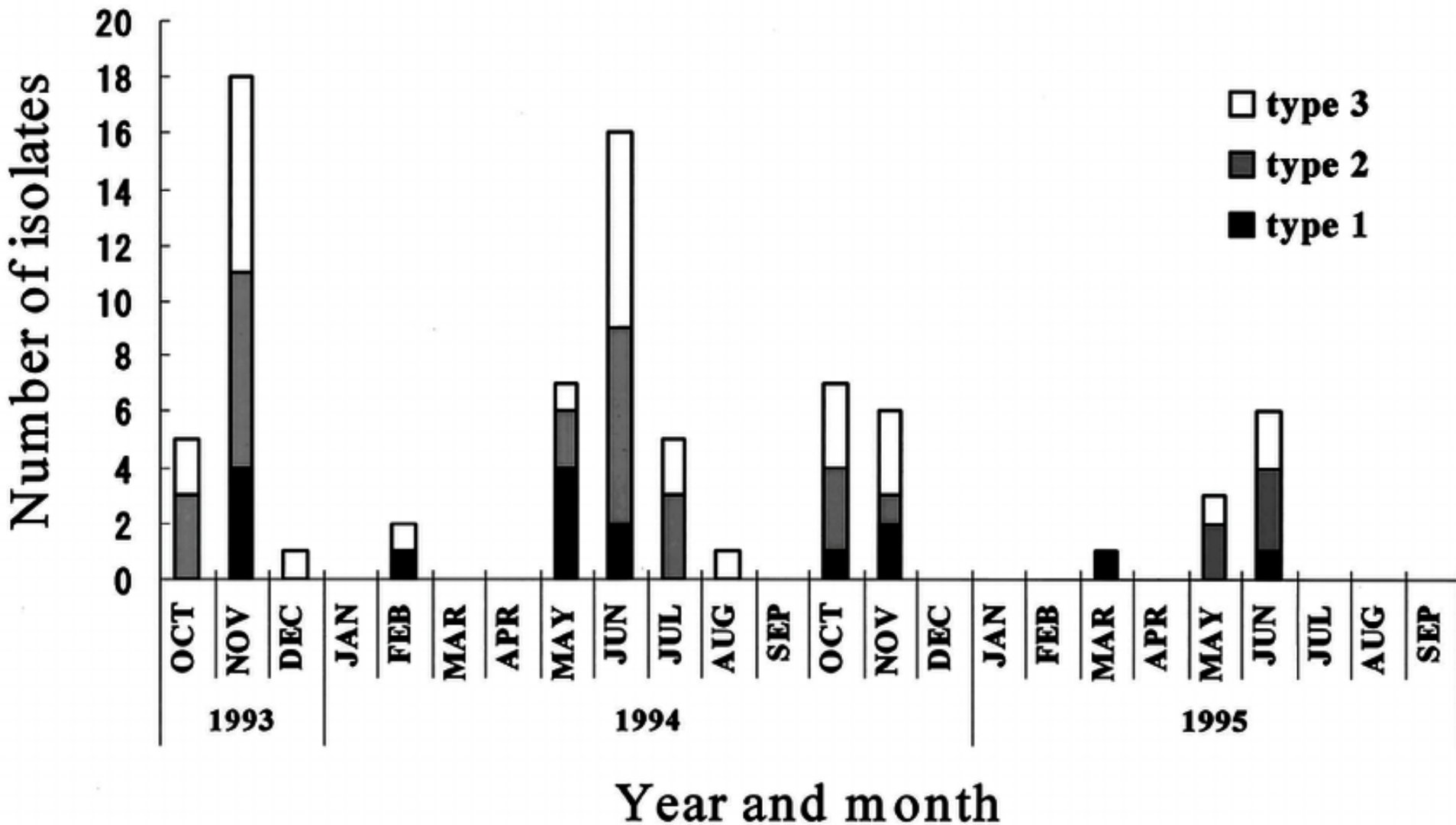
Poliomyelitis



Most people who get infected with poliovirus ($\sim 72 / 100$) **will not have** visible symptoms. An infected person may spread the virus to others **before** and **up to 2 weeks** after symptoms appear.

- **Symptoms** vary from mild **flu**-like symptoms to life-threatening **paralysis**.
- Disease can be spread by respiratory and fecal routes

Polioviruses Isolated from River Water and Sewage



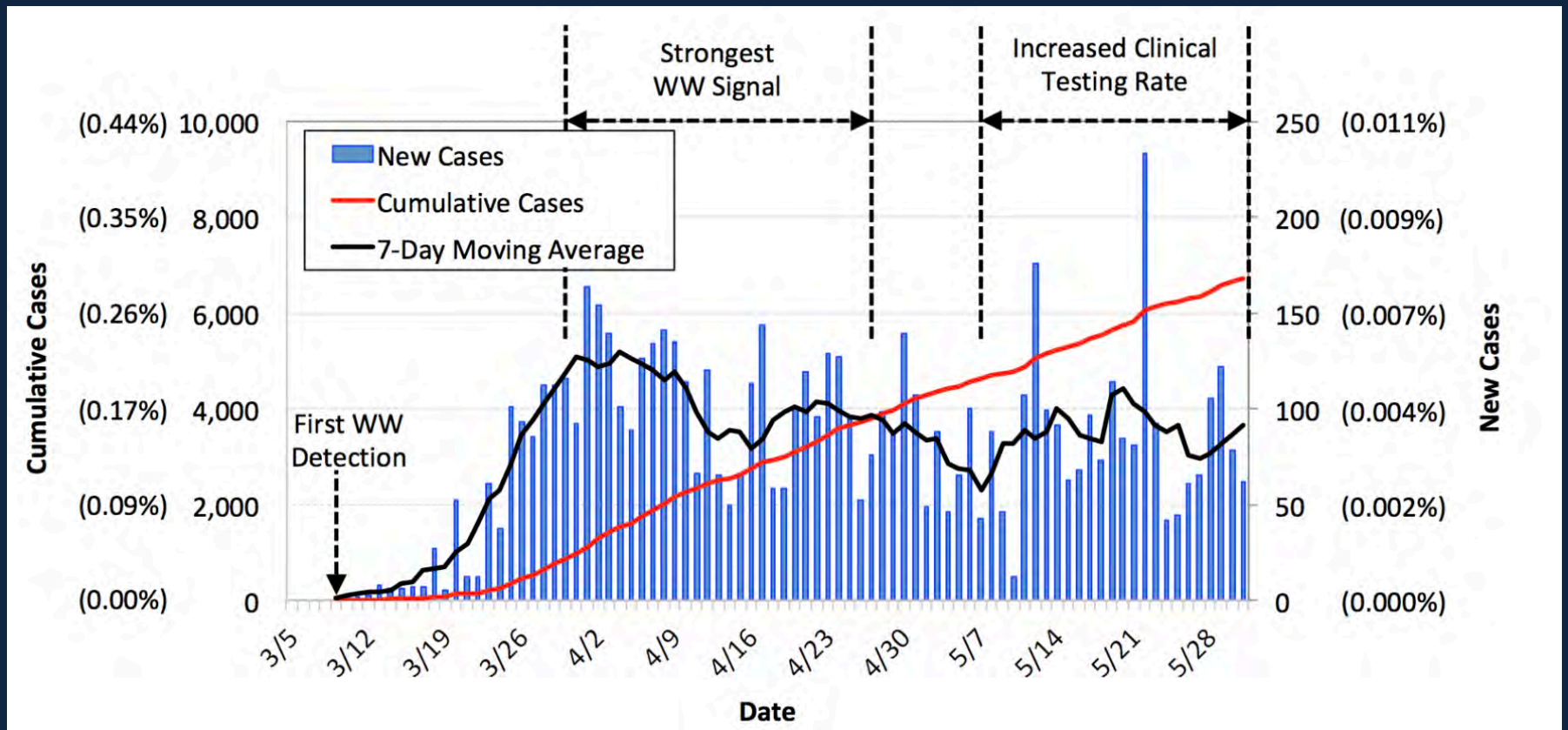
How do you develop a monitoring system that is efficient and relatively inexpensive to track microbes (and illicit drugs)?

- SARS-CoV-2
- Influenza
- Monkeypox
- Candida auris, AMRs, Anthrax
- Other bioterrorism agents
- and?

Vignettes

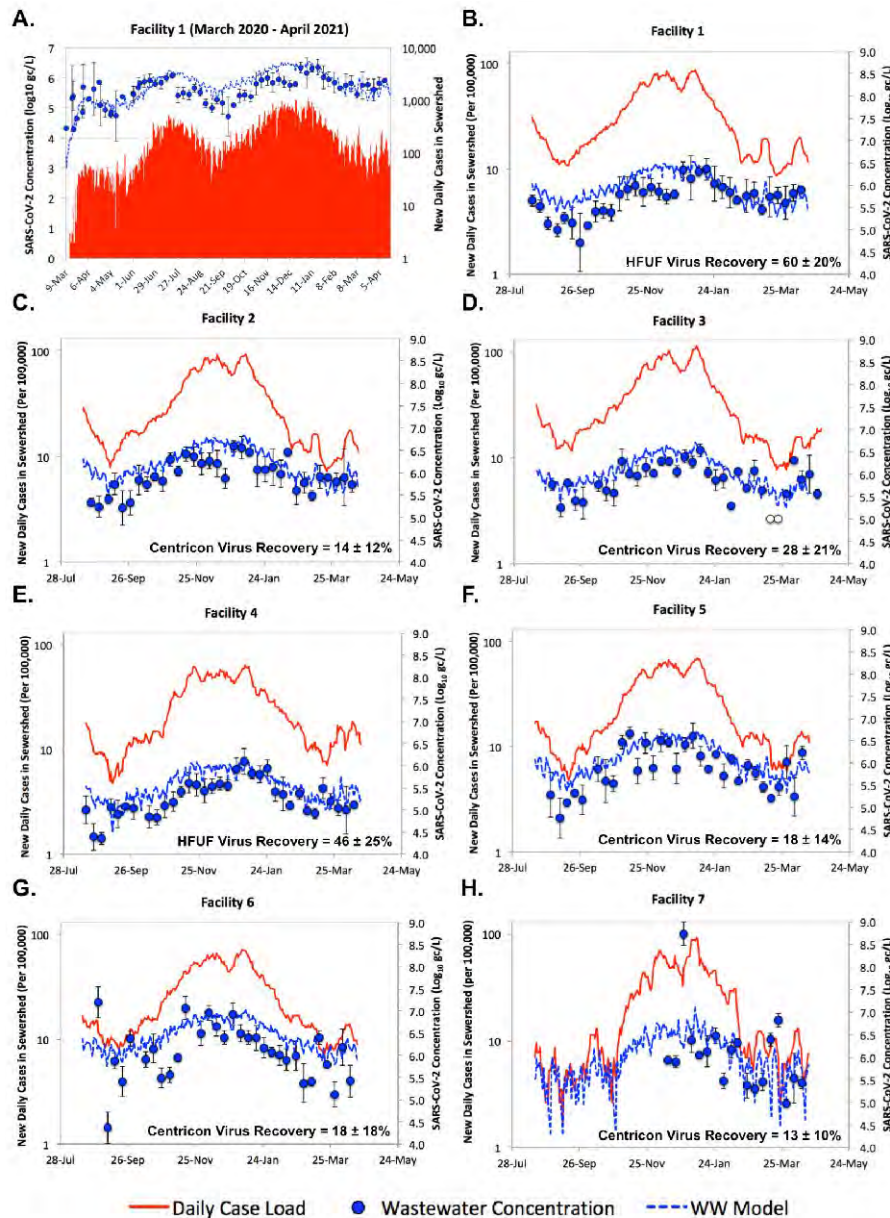
1. Wastewater-based genomics for COVID-19 at the community- and facility-level in Arizona and Nevada
2. Analysis of **actionable** pathogens in a dynamic city:
An Early Warning System

Vignette 1: WGS analysis of wastewater from treatment facilities predicts human infections in NV



Southern Nevada Water Authority: Dan Gerrity and Katerina Papp

WGS analysis of wastewater samples predicts human infections in NV

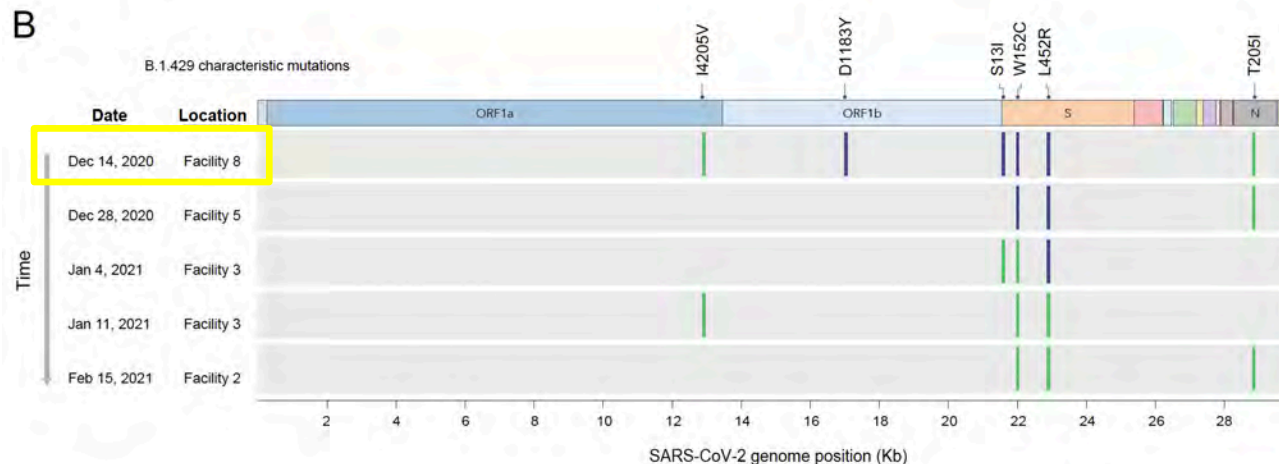
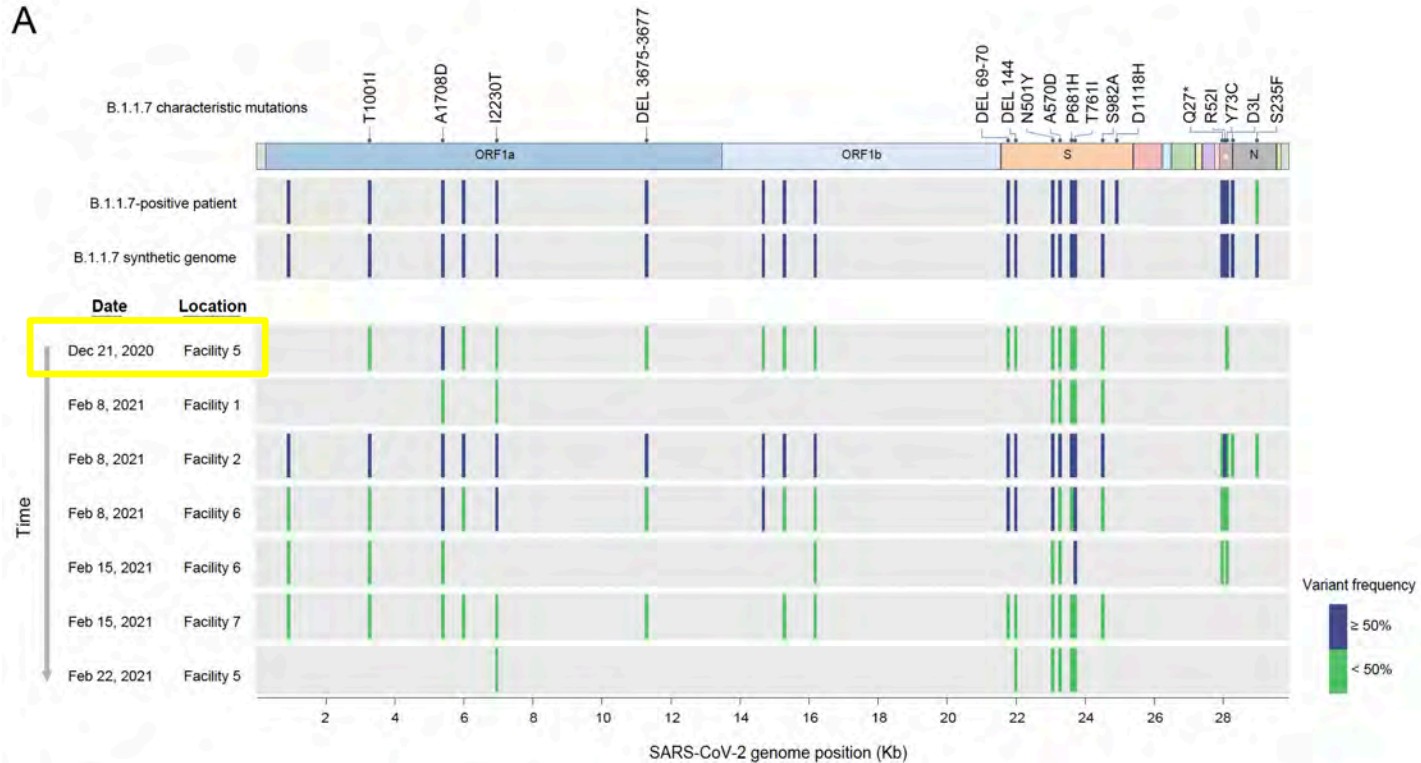


NGS Workflow

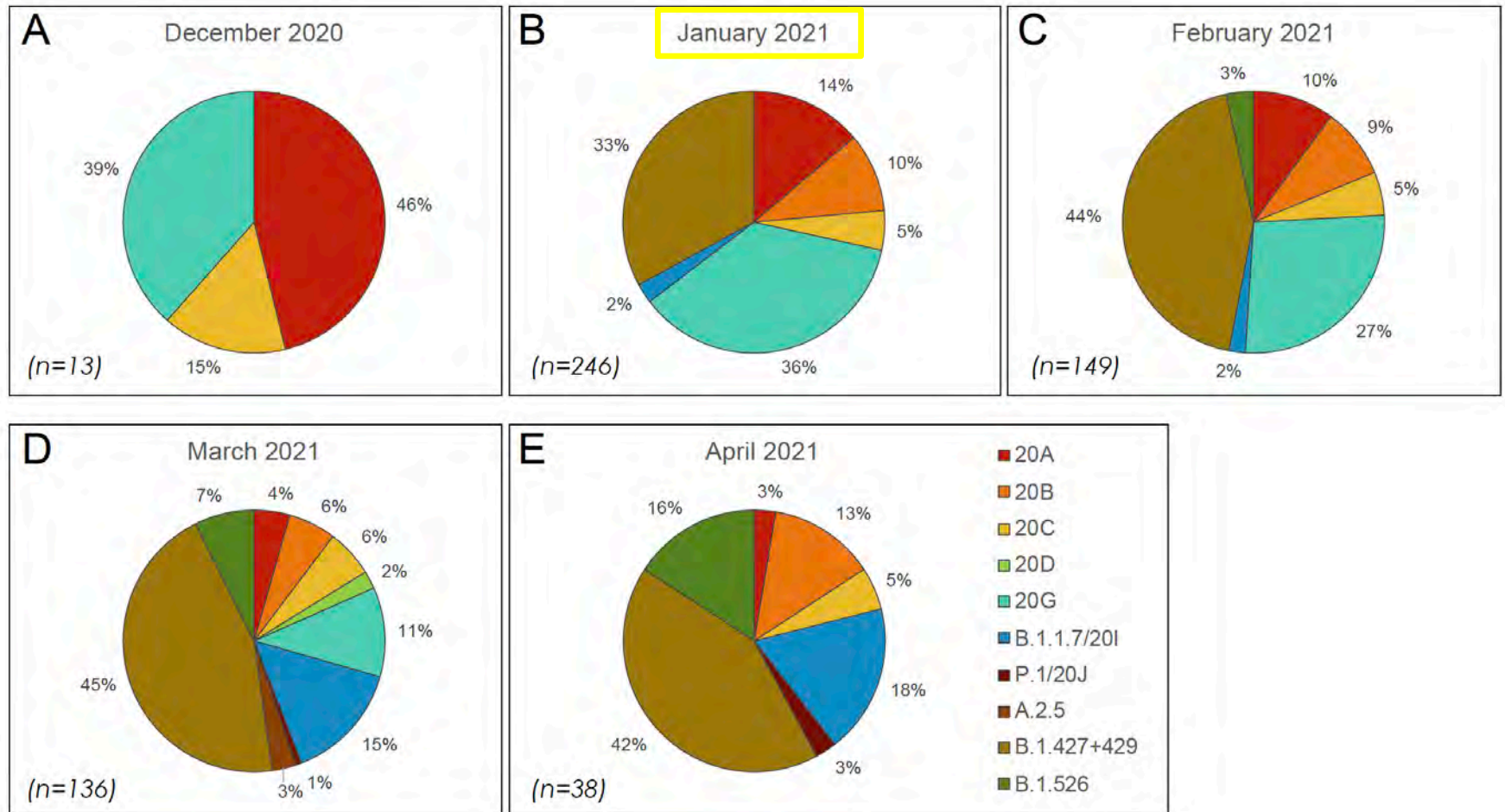
- 1) At least 10ng cDNA
 - wastewater and clinical samples
- 1) Library prep kits – Hybridization based (Agilent & Illumina) vs. Amplicon based (Paragon, Swift, and Qiagen)
- 2) Sequence on Nextseq 500: mid or hi output flowcell
- 3) Metrics – Achieve >50X depth and >80% coverage. Minimum threshold of 5% variant frequency.

Figure 1

Sequencing of **wastewater** from Southern Nevada



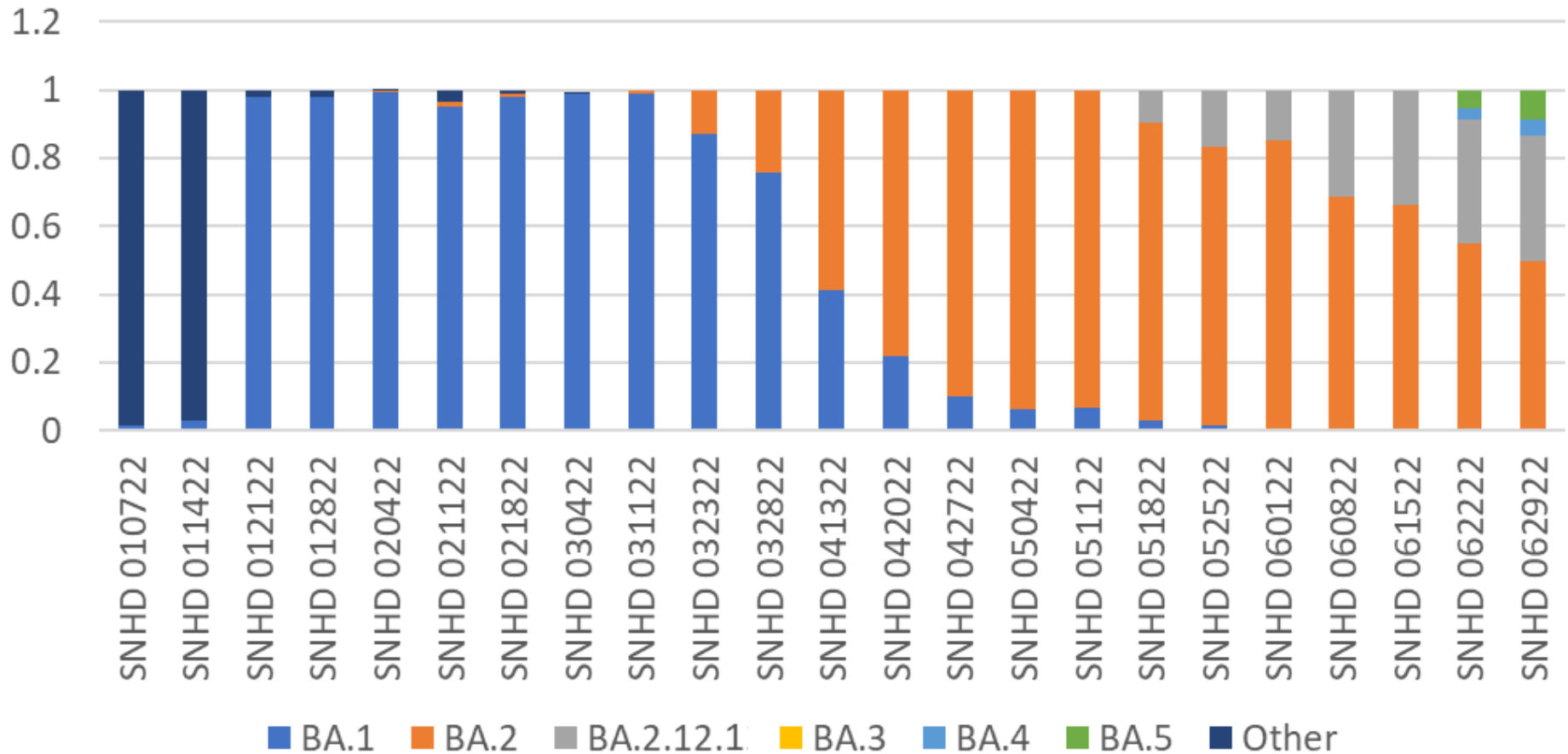
Sequencing of **clinical cases** in Southern Nevada



* WGS analysis of wastewater samples predict emergence of Delta, Mu, and Lambda human infections in NV

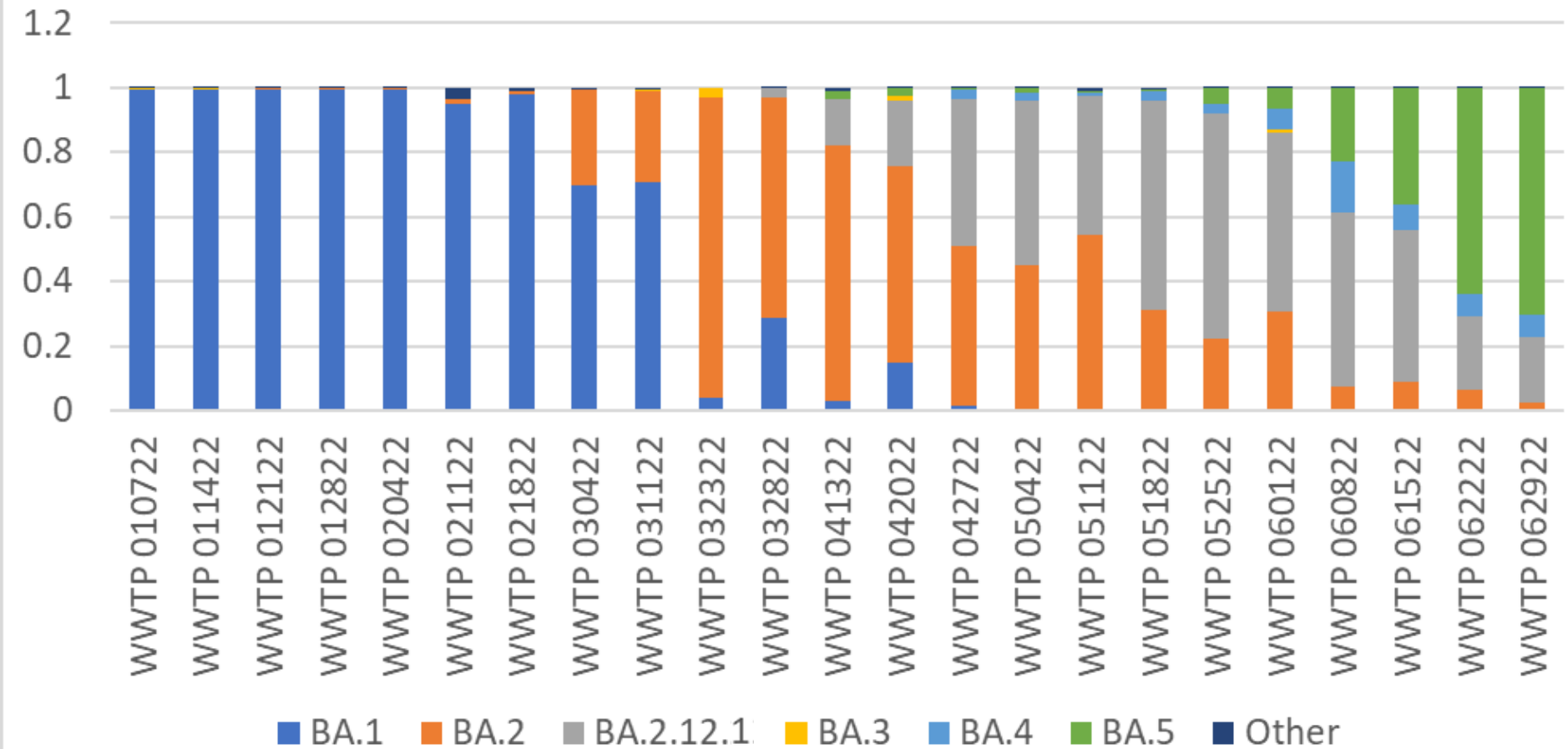
Did we get lucky?

Variant Abundances of Reported Clinical Cases



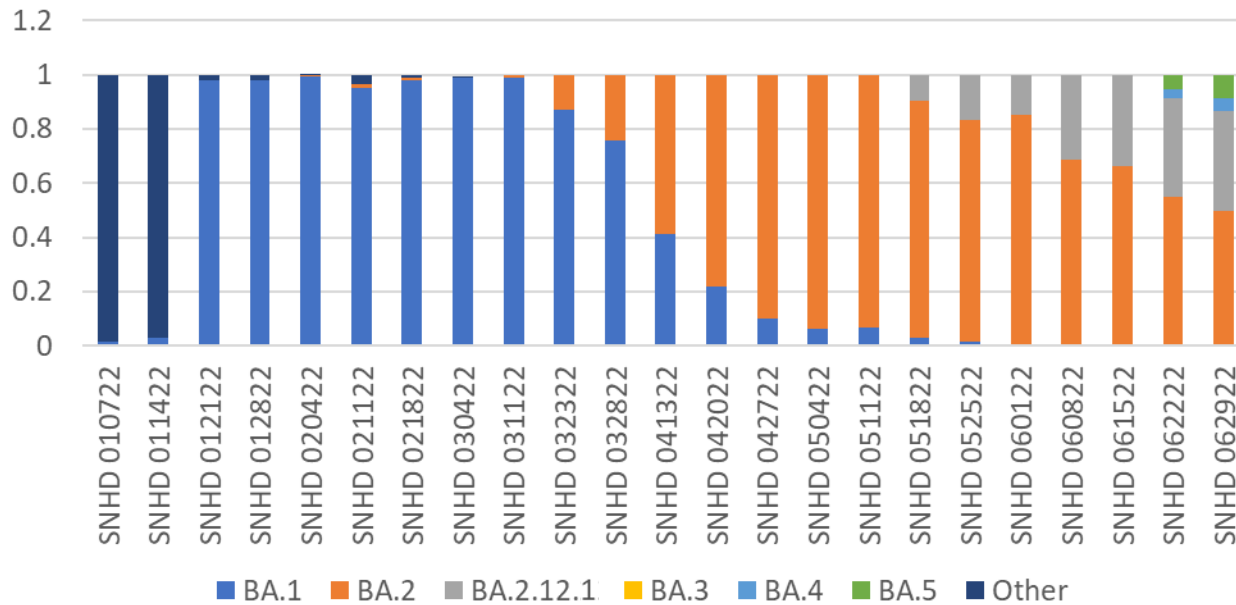
Public Health Surveillance – Southern Nevada

Avg Estimated WWTP Variant Abundances



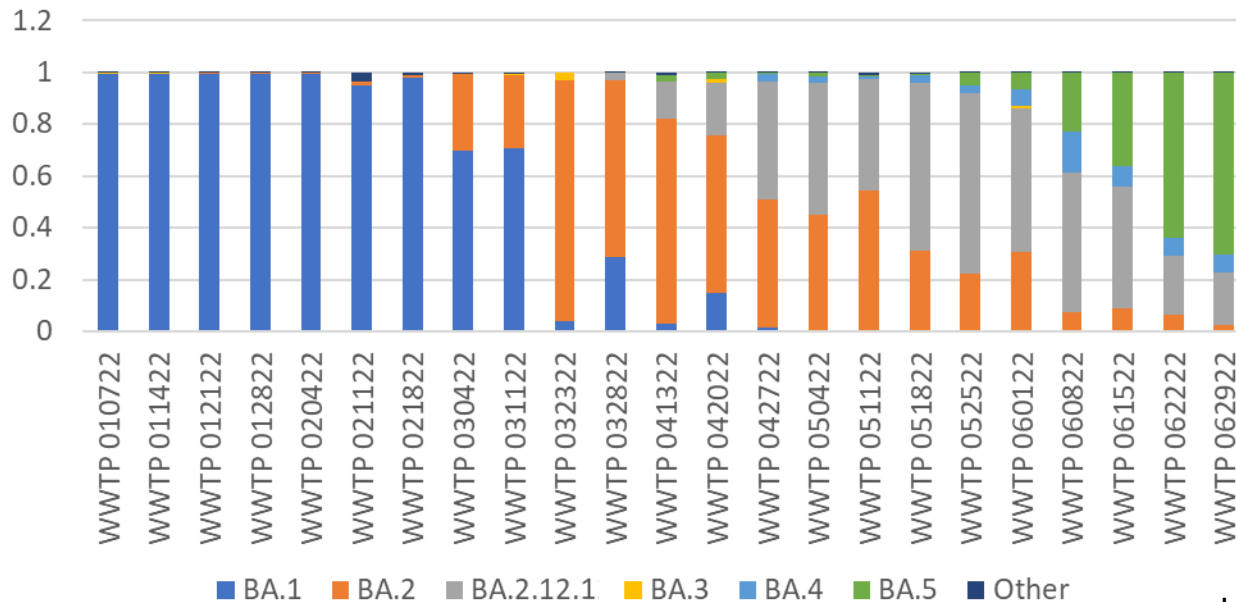
Wastewater Surveillance – Southern Nevada

Variant Abundances of Reported Clinical Cases



Public Health

Avg Estimated WWTP Variant Abundances



Wastewater



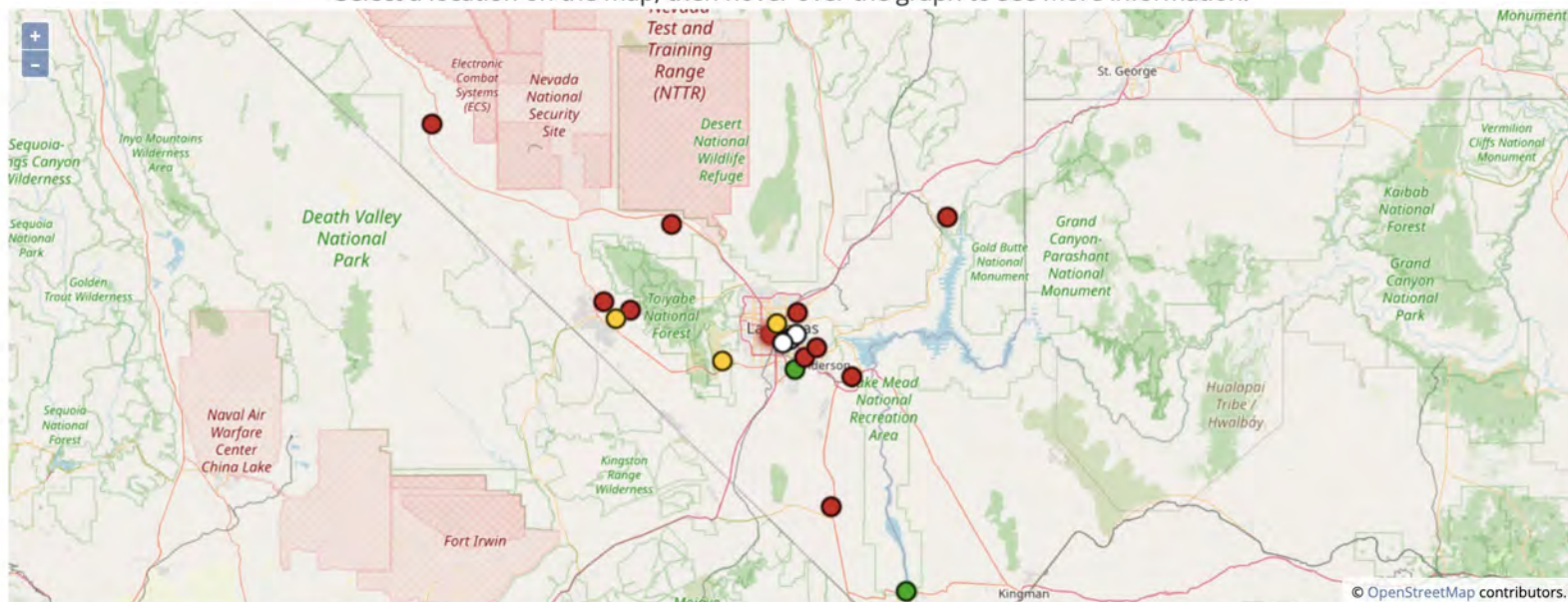
WELCOME TO THE NV EMPOWER PROGRAM

NV Enabling the Management of Public health Outcomes through Wastewater Resources

GET STARTED

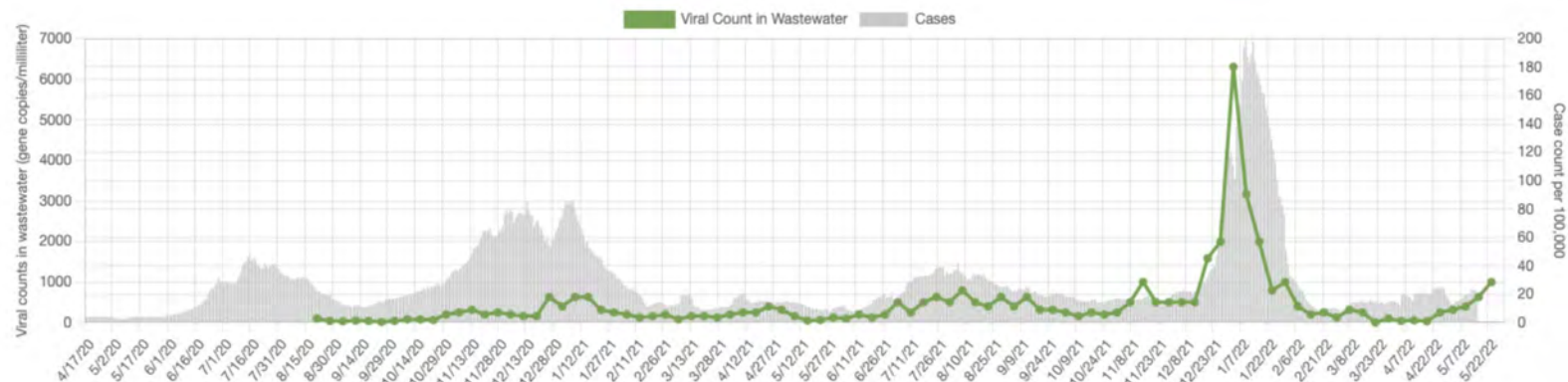
Map of Southern Nevada

Select a location on the map, then hover over the graph to see more information.



● Decreased viral Concentration |
 ● No change in viral concentration |
 ● Increased viral Concentration |
 ● No data available

Clark County



Treatment Facilities



VS.

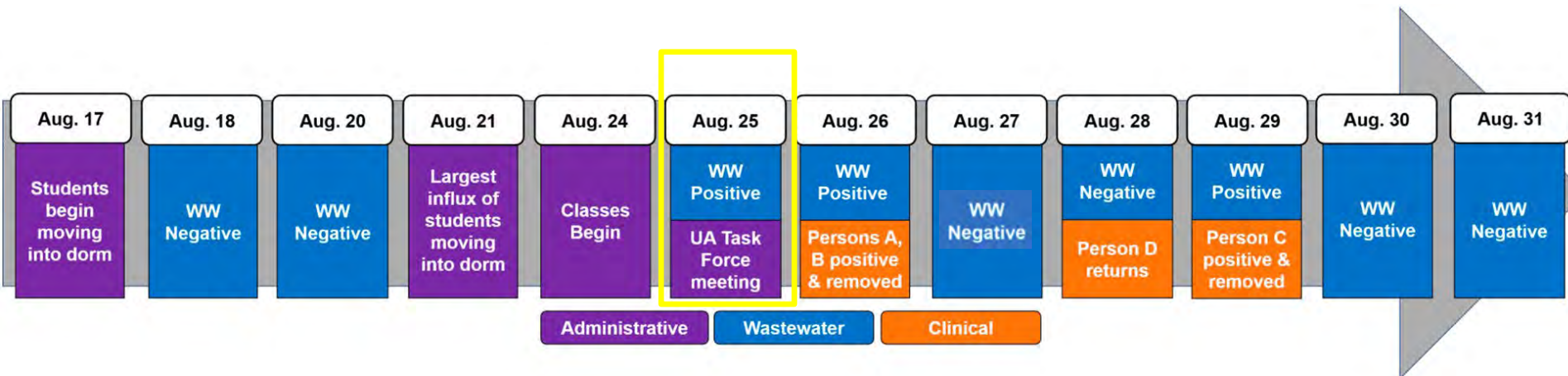


Manholes



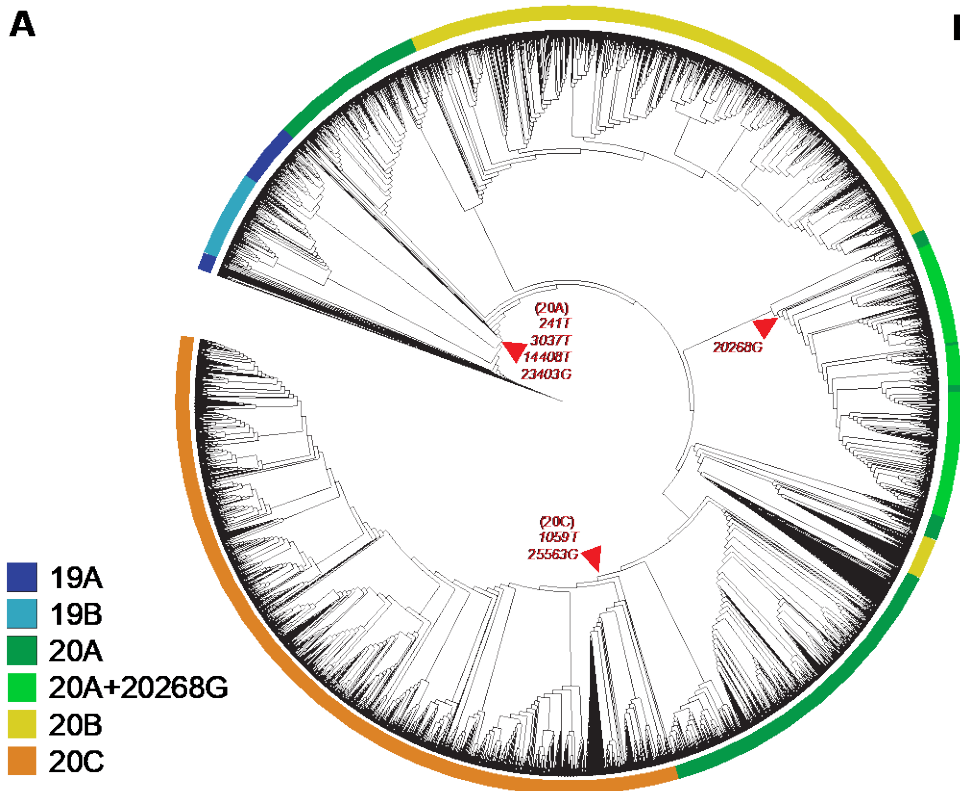
Stormwater

Averting an outbreak at an AZ dormitory - Fall 2020

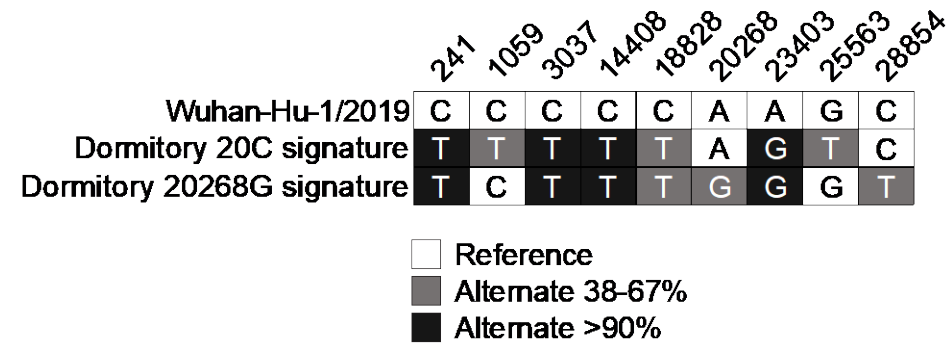


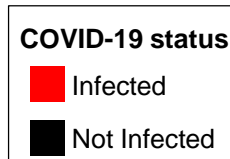
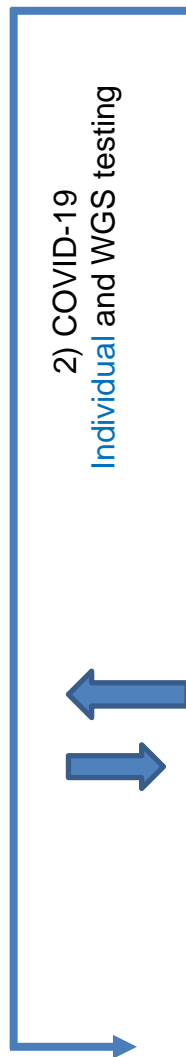
Identification of wastewater SARS-CoV-2 genome(s) from a university dormitory

A

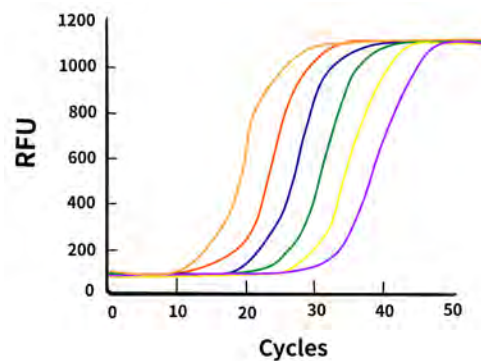


B

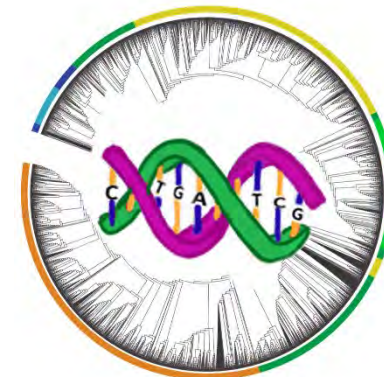




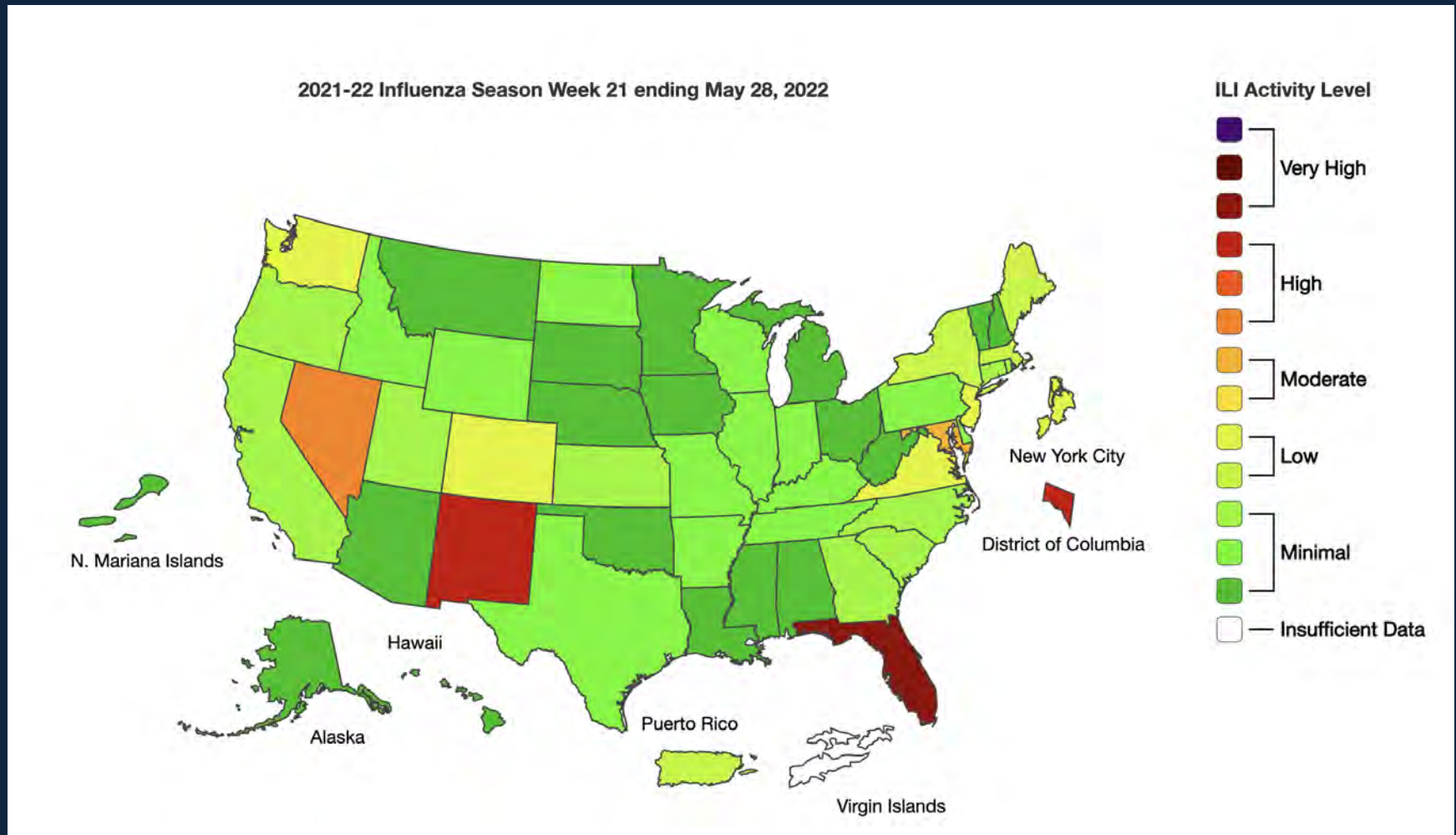
Quantitative PCR



Whole Genome Sequencing (WGS)

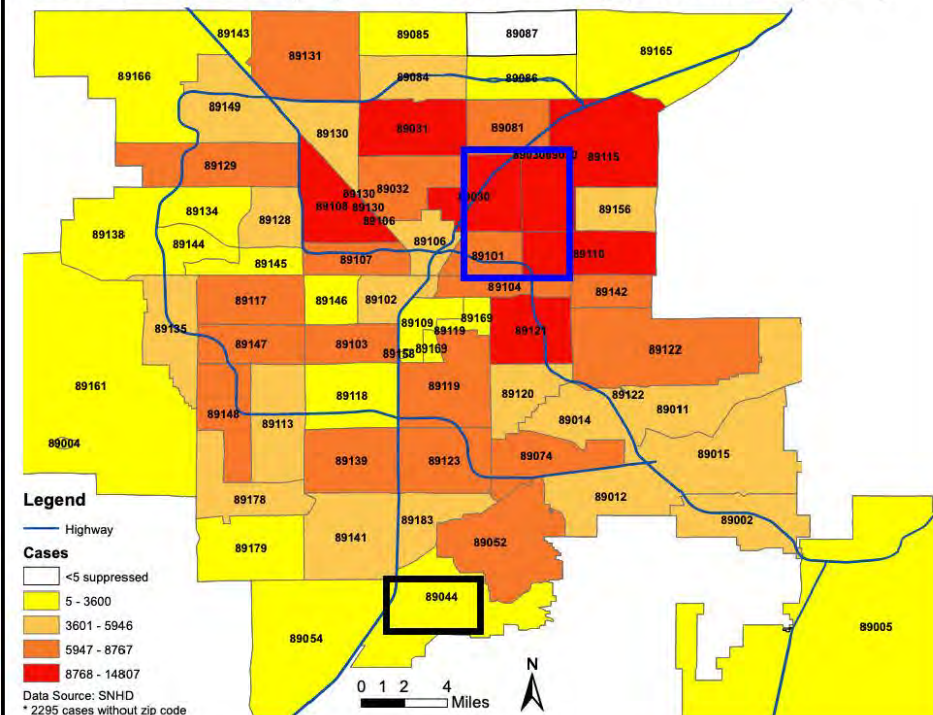


Vignette 2: Analysis of **actionable** pathogens in a dynamic city – and interventions

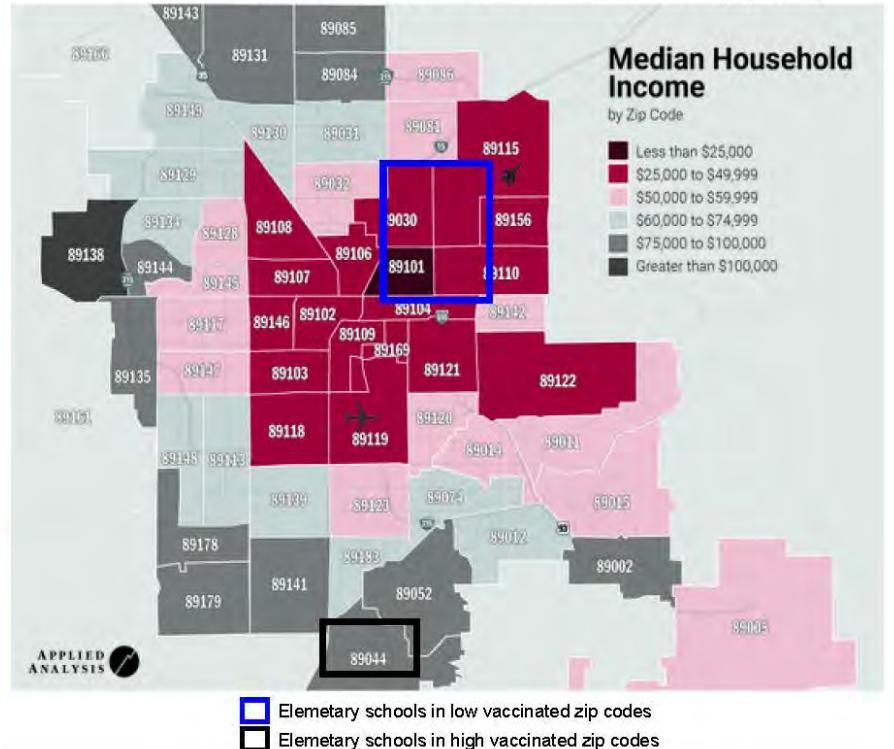


Emergence of SARS-CoV-2 health disparities in vulnerable populations revealed through sewage - and potential interventions

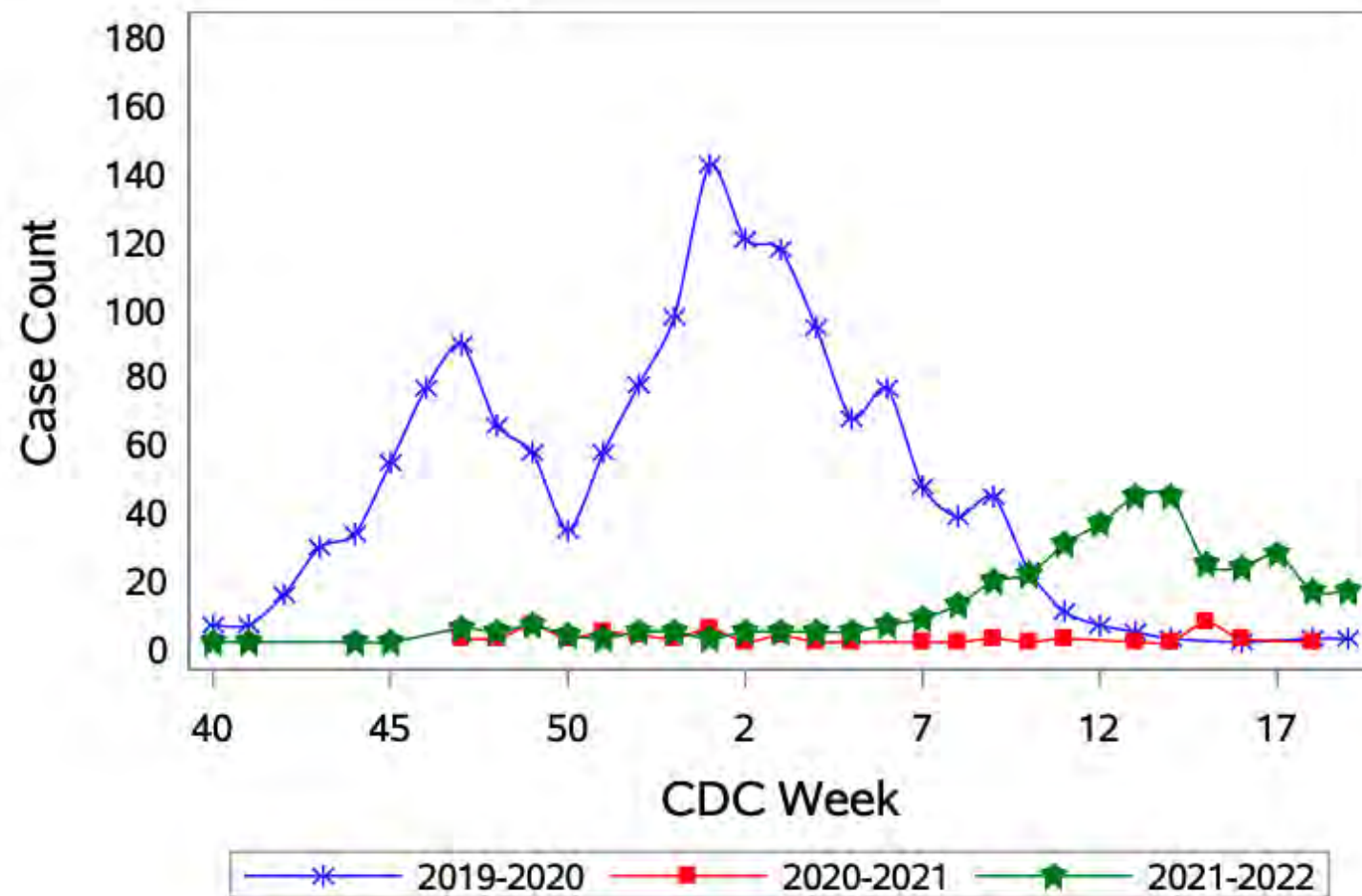
A. COVID-19 cases by zip code in Clark county (November 25, 2021)



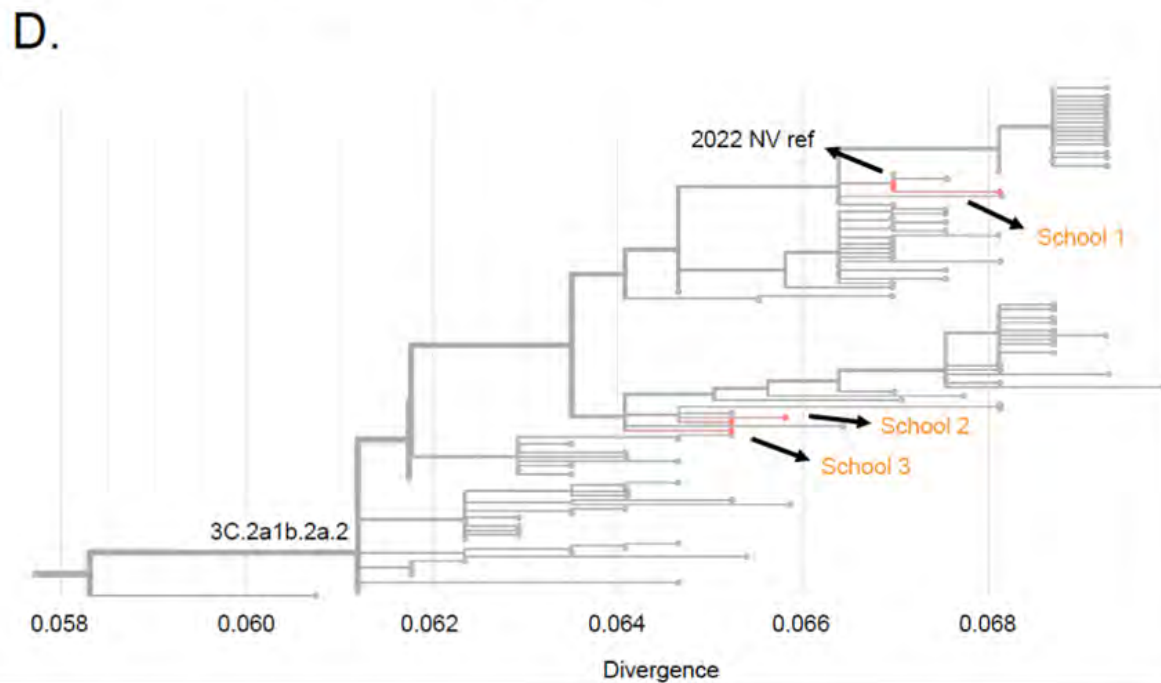
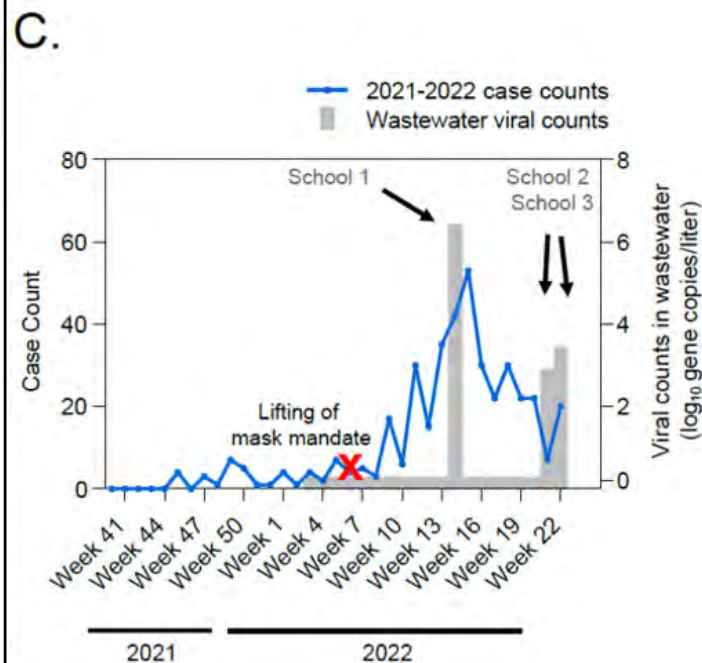
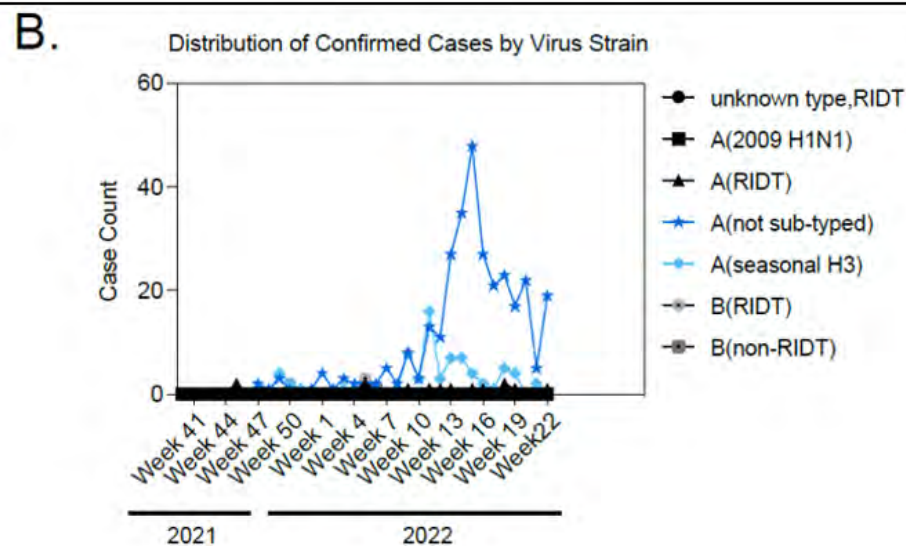
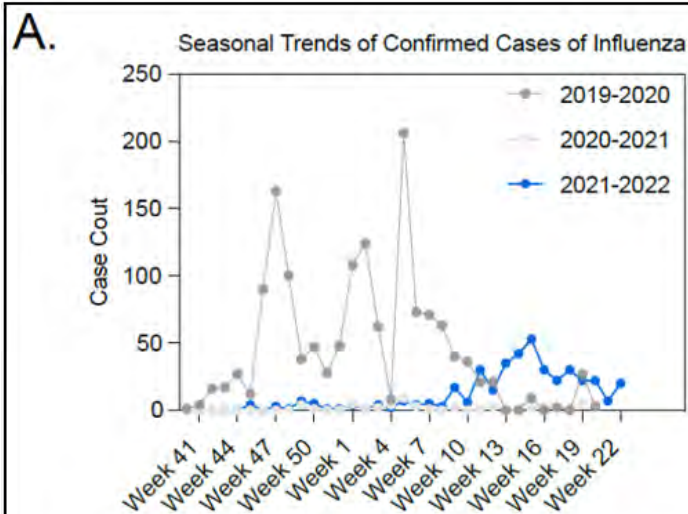
B. Median household income levels in Clark county (2021)



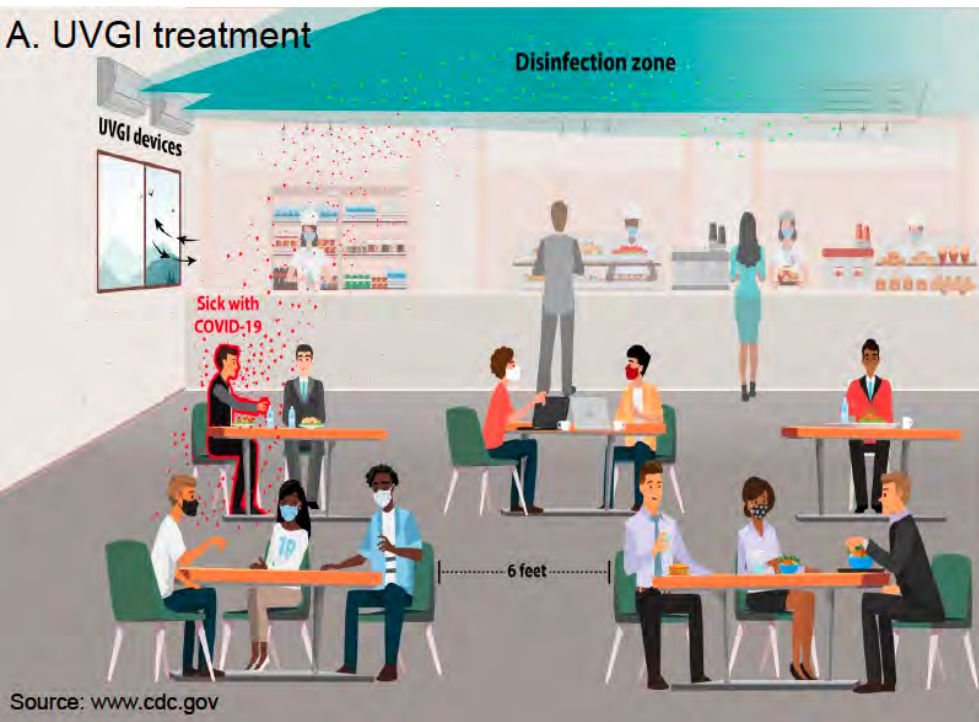
Public health surveillance of Influenza



Total Confirmed Cases*(Week 40 - 20):
1489(2019-2020) 53(2020-2021) 376(2021-2022)



Interventions - Ultraviolet Germicidal Irradiation (UVGI)



B. Schema

UVGI-treated schools & Control schools

Exposure: COVID-19, RSV, Influenza

Aim 1: Data Collection - features

- 1) Wastewater viral counts from school
- 2) Wastewater viral counts from zip code
- 3) Case counts from school
- 4) Case counts from zip code

- additional variables: vaccination rates, income levels, temperature, humidity

Aim 2: Whole genome sequencing - mutational rate?

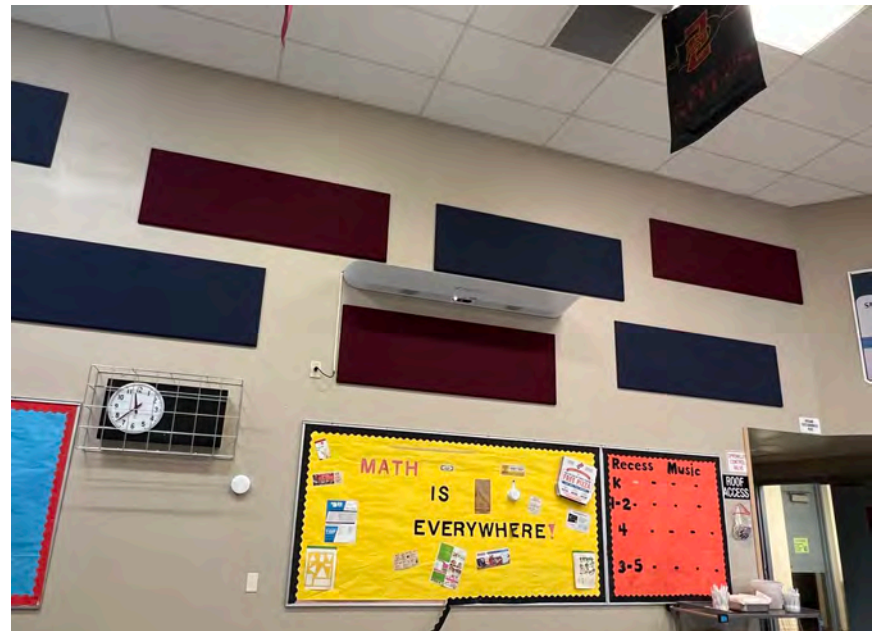
Time-sensitive opportunity to study:

**** 6,000 students > 60% minorities**

**** 2 year impact: 320,000 students**

Biosafety Technology for the Modern World

A continuous, autonomous disinfection ecosystem for indoor shared spaces.



COVID-19 Positive Cases

Districtwide Total Cases

24,598

School Staff

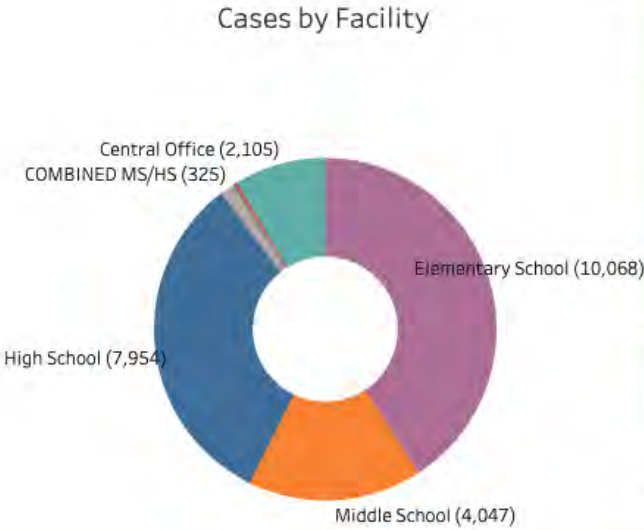
7,315

Central Staff

1,995

Students

15,288



Facility

Trustees

Zip Code

Districtwide Positive Cases

Elementary School Middle School High School COMBINED MS/HS Adult School Ungraded Central Office

Location Type
(All)

Location Name
(All)

Yesterday
0

This Week
112

This Month
51

School Year 2021-2022
23,969

Last Updated on
June 2, 2022

- The data presented may not always match the most recent reports released by local health jurisdictions or the Nevada Department of Health and Human Services.
- Data is based on internally confirmed and self-reported COVID-19 positive lab results (both PCR and rapid are included).
- Districtwide total cases are cumulative cases tracked since July 1, 2021 for the purpose of this Dashboard.
- School year 2021-2022 includes testing on Aug 9, 2021 through May 25, 2022.
- Student cases are reported based on self-reported information or SNHD confirmed cases. There is no distinction between face-to-face, hybrid, or virtual instruction participants.
- Individual statistical counts lower than 10 are intentionally withheld for privacy purposes. '<10' will display instead of the count until the data reaches 10 or more cases.
- Zip Code tracking is based upon the school or office location where the student or staff member is assigned.
- All data are provisional and subject to change. Data is updated regularly, but is not real-time, to allow for data verification and integrity.

Results

Control		UVGI
21-Jan	Not detected	44
26-Jan	711	270
28-Jan	114266	Not detected
31-Jan	Not detected	276
2-Feb	620	Not detected
4-Feb	Not detected	Not detected
7-Feb	Not detected	Not detected
9-Feb	Not detected	Not detected
11-Feb	Not detected	Not detected
14-Feb	Not detected	81616
16-Feb	Not detected	Not detected
18-Feb	Not detected	Not detected
23-Feb	Not detected	Not detected
25-Feb	564	Not detected
28-Feb	Not detected	Not detected
2-Mar	Not detected	Not detected
4-Mar	Not detected	Not detected
7-Mar	Not detected	Not detected
9-Mar	52	Not detected
11-Mar	Not detected	Not detected
16-Mar	Not detected	Not detected
18-Mar	Not detected	Not detected
21-Mar	Not detected	Not detected
23-Mar	Not detected	Not detected
25-Mar	Not detected	Not detected
28-Mar	154	Not detected
30-Mar	Not detected	Not detected
1-Apr	Not detected	Not detected
4-Apr	Not detected	Not detected
6-Apr	Not detected	Not detected
8-Apr	Not detected	Not detected
20-Apr	Not detected	Not detected
22-Apr	1	Not detected
25-Apr	Not detected	Not detected
27-Apr	Not detected	Not detected
29-Apr	Not detected	Not detected
2-May	Not detected	Not detected
4-May	Not detected	Not detected
6-May	Not detected	Not detected
9-May	Not detected	Not detected
11-May	Not detected	Not detected
13-May	Not detected	Not detected
16-May	Not detected	Not detected
18-May	90	Not detected
20-May	Not detected	Not detected
23-May	Not detected	Not detected
25-May	Not detected	Not detected

[illegible]

Control		UVGI
21-Jan	Not detected	Not detected
26-Jan	Not detected	184
28-Jan	Not detected	Not detected
31-Jan	82	Not detected
2-Feb	Not detected	628
4-Feb	Not detected	Not detected
7-Feb	Not detected	Not detected
9-Feb	Not detected	Not detected
11-Feb	Not detected	Not detected
14-Feb	Not detected	Not detected
16-Feb	Not detected	Not detected
18-Feb	Not detected	Not detected
23-Feb	Not detected	12129
25-Feb	113971	Not detected
28-Feb	Not detected	Not detected
2-Mar	Not detected	Not detected
4-Mar	Not detected	Not detected
7-Mar	32	Not detected
9-Mar	Not detected	2858
11-Mar	Not detected	Not detected
16-Mar	Not detected	Not detected
18-Mar	Not detected	Not detected
21-Mar	756	Not detected
23-Mar	Not detected	Not detected
25-Mar	Not detected	Not detected
28-Mar	Not detected	Not detected
30-Mar	Not detected	Not detected
1-Apr	Not detected	Not detected
4-Apr	Not detected	Not detected
6-Apr	Not detected	Not detected
8-Apr	Not detected	Not detected
20-Apr	Not detected	Not detected
22-Apr	6	Not detected
25-Apr	Not detected	Not detected
27-Apr	Not detected	2393
29-Apr	Not detected	Not detected
2-May	107	Not detected
4-May	Not detected	Not detected
6-May	Not detected	Not detected
9-May	Not detected	Not detected
11-May	1	Not detected
13-May	1	39004174976
16-May	481	372
18-May	Not detected	202
20-May	62250	Not detected
23-May	Not detected	Not detected
25-May	Not detected	16630

UVGI Treatment vs. None

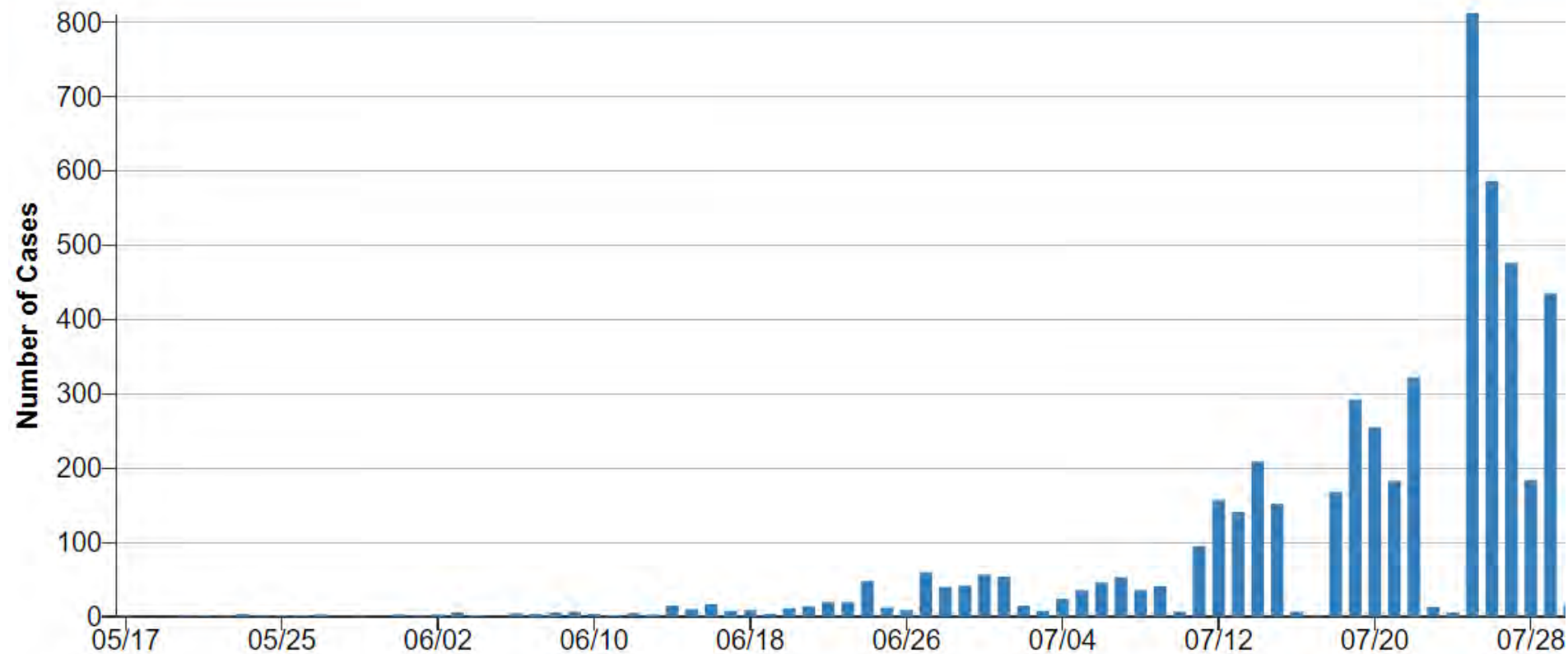


Monitoring shelters, schools, dorms, hotels, stadiums, etc.

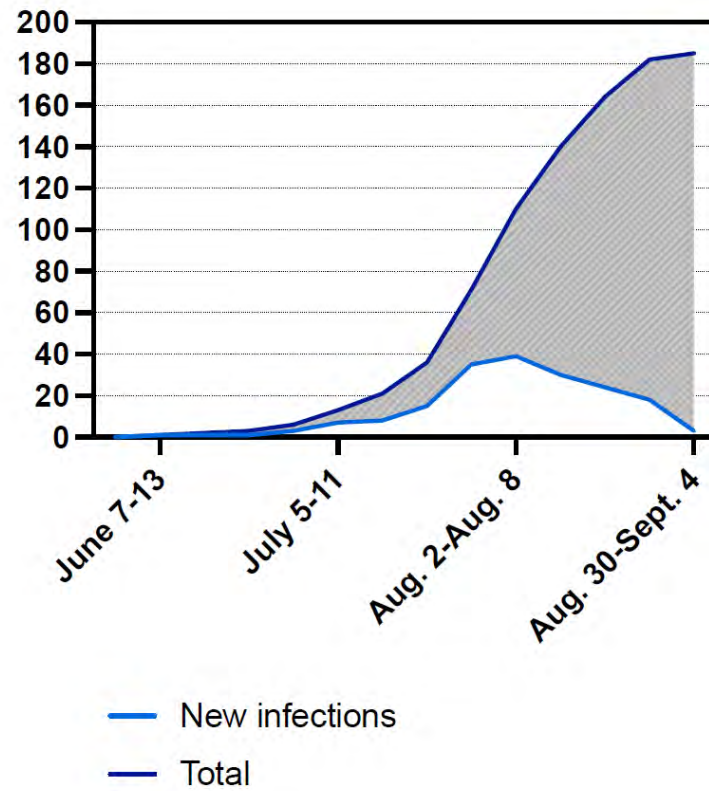


Detection of Monkeypox

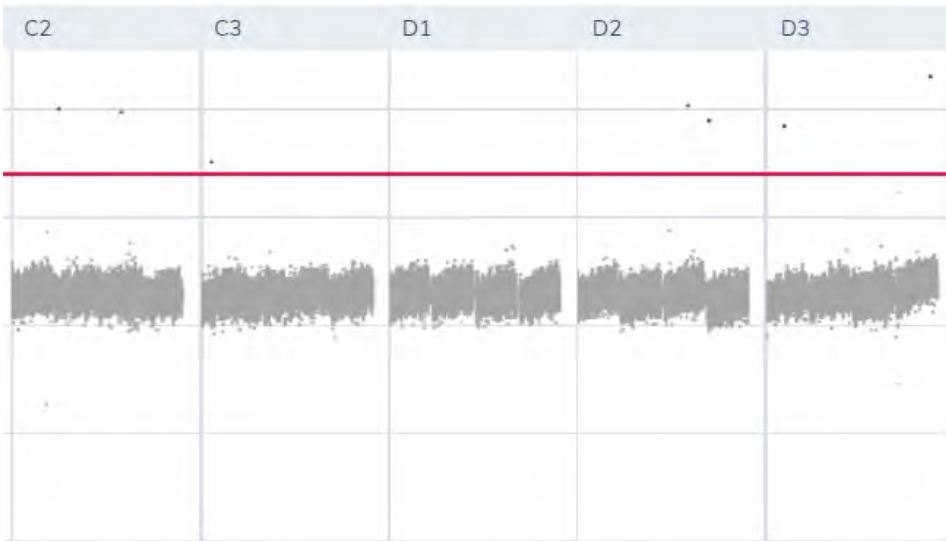
U.S. Monkeypox Case Trends Reported to CDC



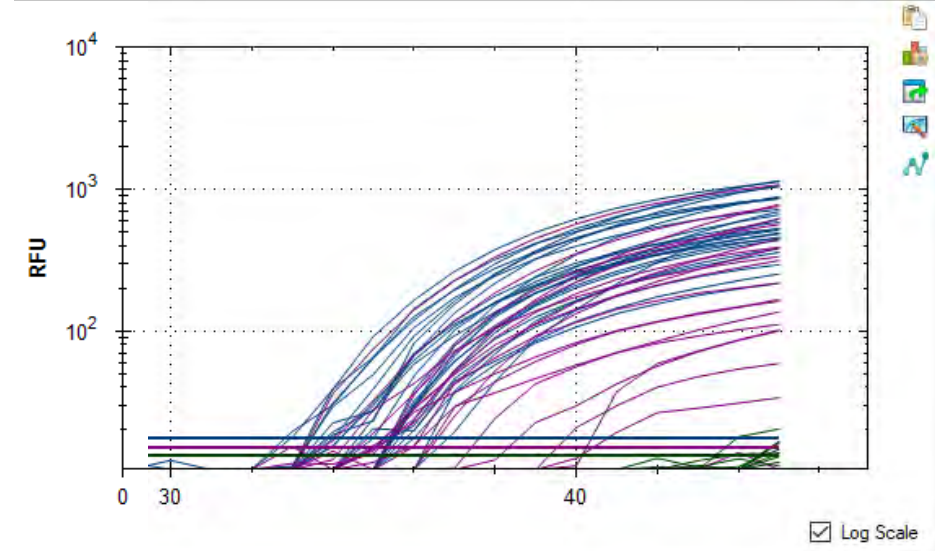
Monkeypox infections in Clark County



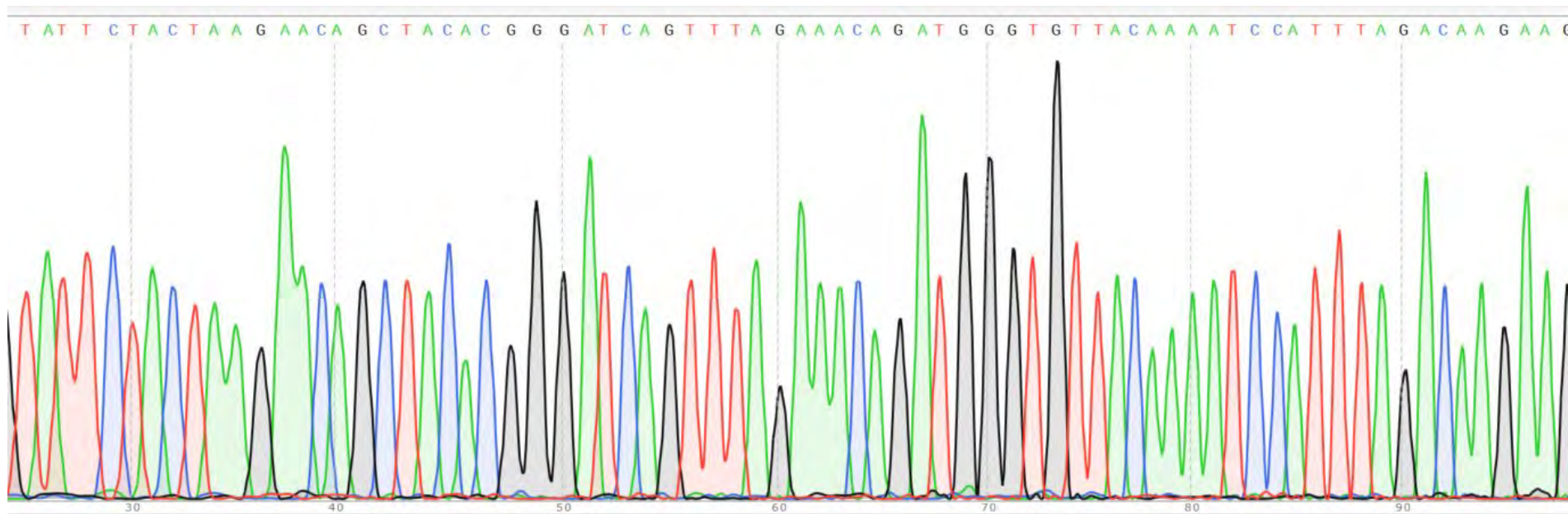
Wastewater monkeypox Digital PCR



Wastewater monkeypox Quantitative PCR



Sanger Sequencing – monkeypox PCR product



Summary and Future Directions

- PCR and WGS can help determine viral load and identify variants
- Collaboration with community partners (**really essential**) to continue and grow surveillance program
- Extend program to monitor microbial content and analytes/metabolites in both urban and rural communities

Engage the media to share your message



How UNLV researchers can use sewage to help inform public health policy

