



# Virginia Wastewater Surveillance Program: Community of Practice Meeting

WWS Team

VDH | Office of Environmental Health Services

October 26, 2022



# Agenda

- ☐ Updates & Funding Opportunities

- ☐ Topic(s) of Interest:

  - ☐ Optimizing and Validating a Monkeypox Assay

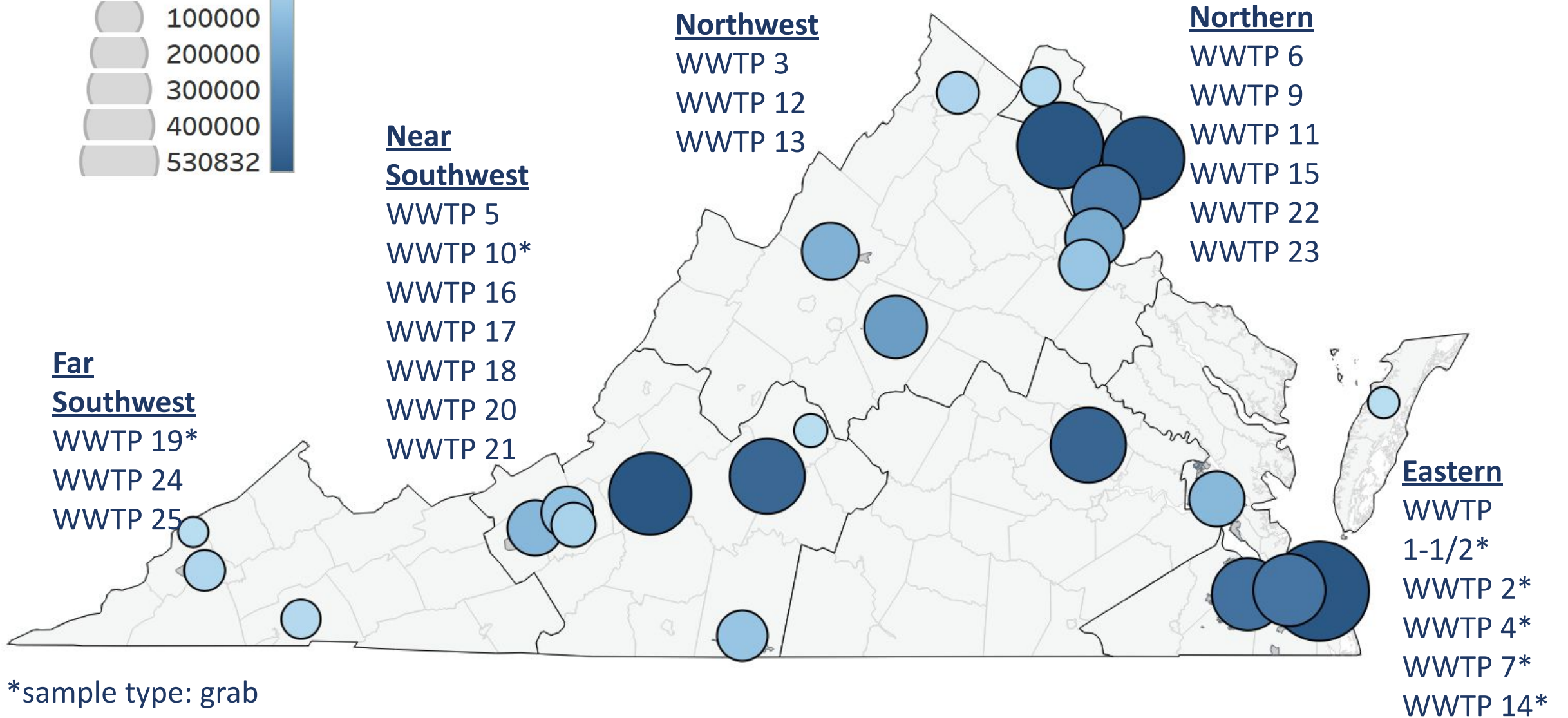
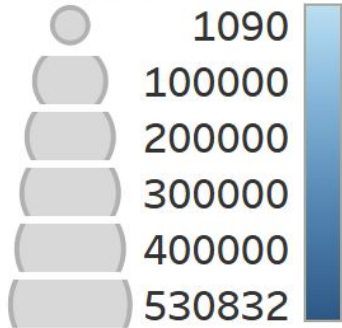
    - for surveillance in Hampton Roads, VA

- ☐ Open Discussion

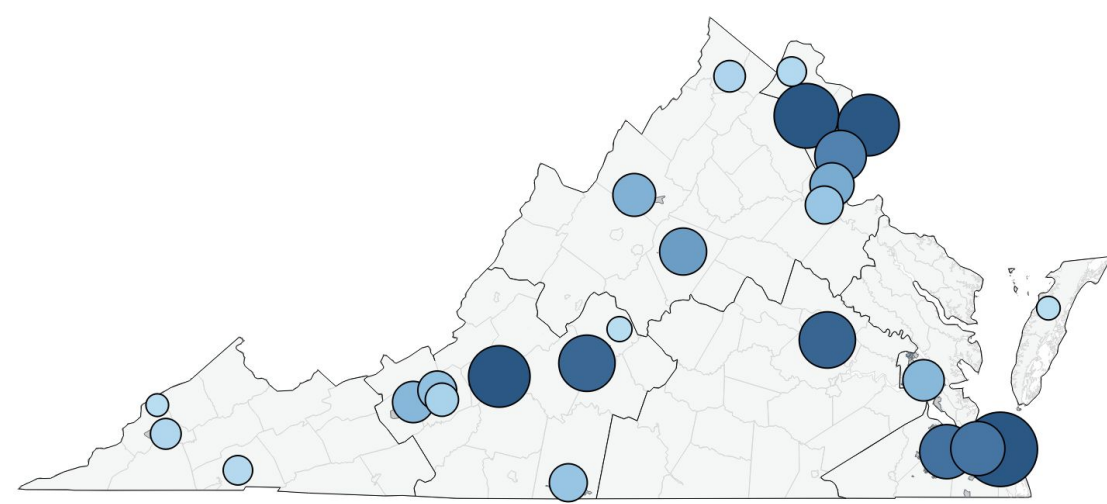


# Sentinel Monitoring Facilities

## Population



# Program Updates



## ❖ SARS-COV-2 MONITORING

- Total 27 wastewater treatment plants
  - 14 sampling *twice* weekly
  - 13 sampling *once* weekly
- *Still enrolling (up to 40 sites)*
- **Internal Dashboard Available!**

## ❖ VARIANT SEQUENCING

- 20 sites weekly (rotating some sites)

# Funding Opportunities

- ❖ **New Funding Received from CDC (BP4)**
- ❖ **Localized Projects**
  - Funding available under Localized Monitoring Program (LMP)
    - Enrolled several LMP SW Virginia sites into SMP
    - Sponsoring Local Projects in Roanoke and Chesapeake area
- ❖ **CDC-Biobot Commercial Sampling**
  - 15 sites currently enrolled from Virginia
  - Added Monkeypox recently
  - Sampling: twice/week
- ❖ **WastewaterSCAN**
  - A National effort based at Stanford University





# Status Report: Monkeypox and Polio

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- ❖ Currently not analyzing for these targets in WW samples in VA
- ❖ Interested in Monkeypox, but not Polio at this time!
  - HRSD can support Monkeypox analysis
  - Working on redirect of funding
- ❖ CDC has created 2 Centres of Excellence to provide guidance on Polio and Monkeypox:
  - East - Houston
  - West - Colorado
- ❖ For more information on Polio, please visit: [Implementation of Poliovirus Containment in the US | CDC](#)

# Polio Surveillance in Virginia

- **Syndromic surveillance**

- Using syndromic surveillance data from ED visits to monitor for potential cases of poliomyelitis based on chief complaint and discharge diagnosis.
- No visits of interest have been detected yet

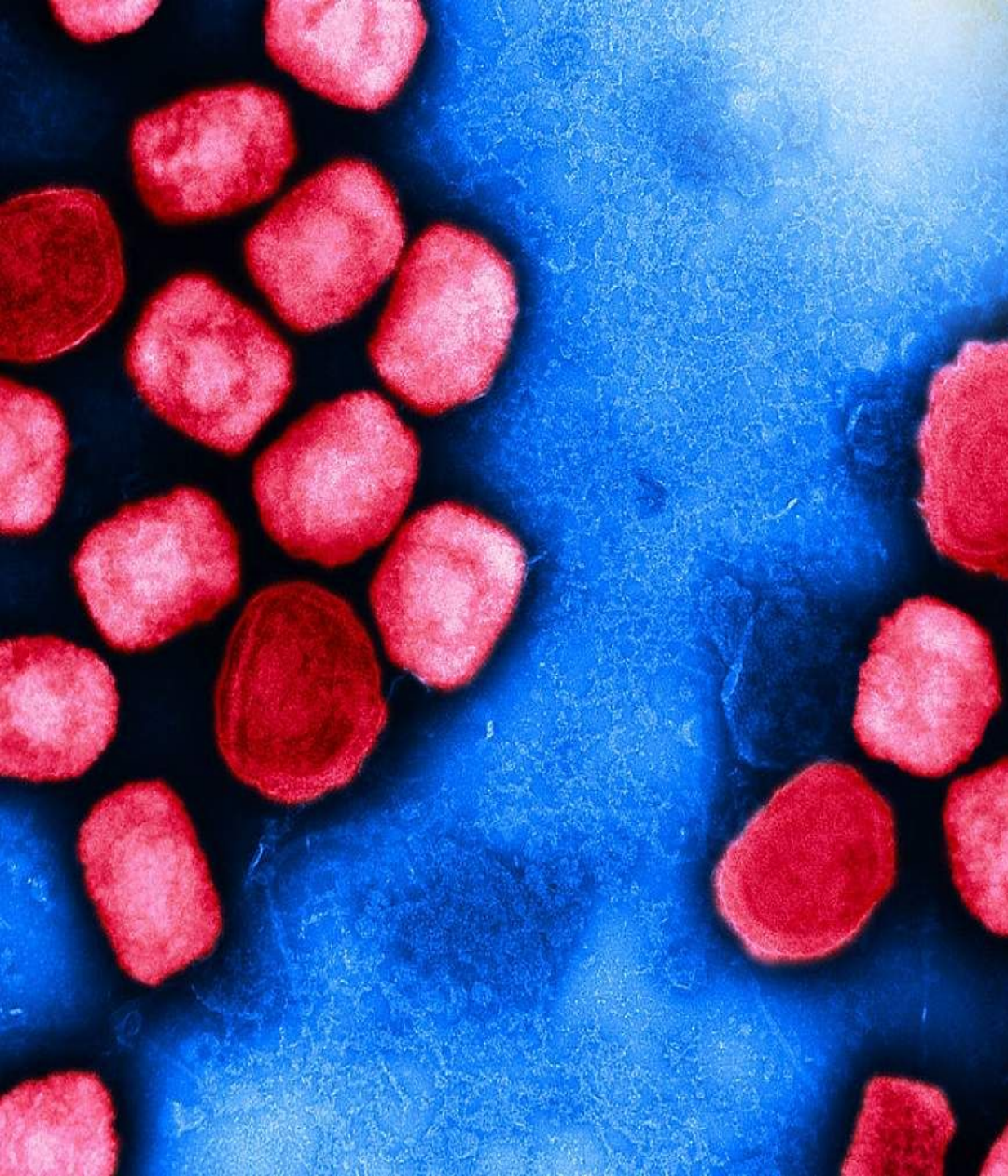
- **Wastewater surveillance**

- VDH is assessing the capacity of wastewater surveillance program to accommodate poliovirus testing.
- Working closely with CDC and Office of Epi for the directions.



[Source](#)





# **Optimizing and Validating a Monkeypox Assay for Surveillance in Hampton Roads, VA**

**Dr. Raul Gonzalez**

**Research Scientist**

**Hampton Road Sanitation District (HRSD)**

**Virginia Beach, VA**



Optimizing and Validating a  
Wastewater Monkeypox Assay  
10/26/22



- **Core Focus Area**

- Microbial Source Tracking
- Environmental Micro Research on Receiving Water Dynamics
- Wastewater Surveillance

- **Matrices**

- Recreational Waters, Stormwater, Biosolids, Wastewater, Water Reuse, Shellfish

- **Capabilities**

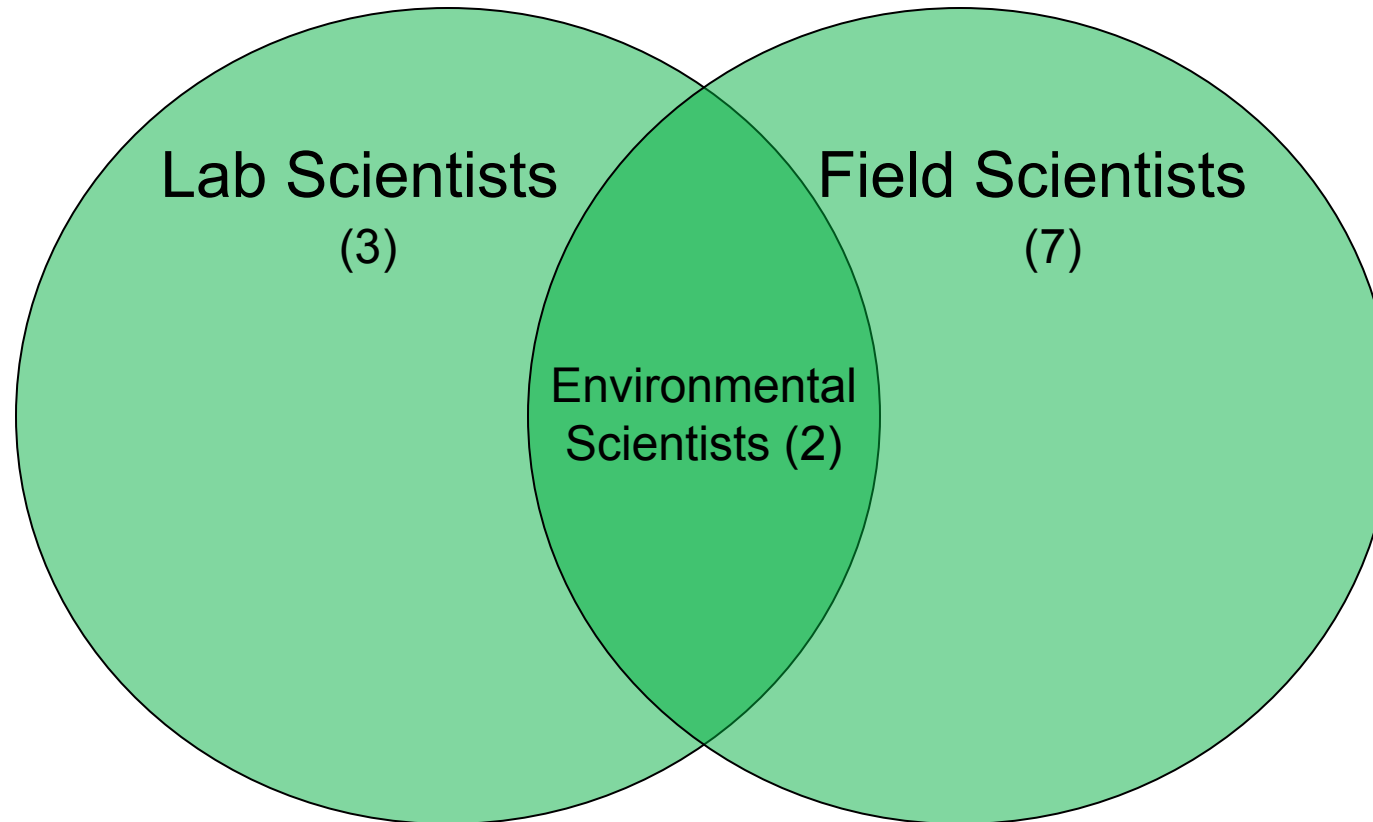
- Digital PCR
- Quantitative PCR
- Sequencing
  - Illumina iSeq 100
  - Nanopore MinION
- Culture:
  - Traditional FIB
  - Coliphage
  - GB-124



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## Pathogen Program cont.

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Why is HRSD monitoring?

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# Regional Viral Load, Hospitalizations, and Deaths

### Aggregate Viral Load for HRSD Treatment Facilities



### Most Recent 8 Weeks



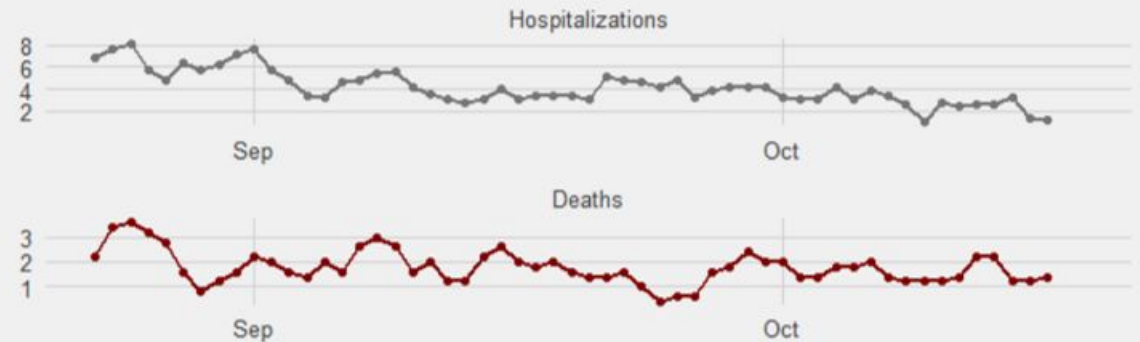
### COVID-19 Hospitalizations and Deaths

Hampton Roads 5 Day Rolling Mean

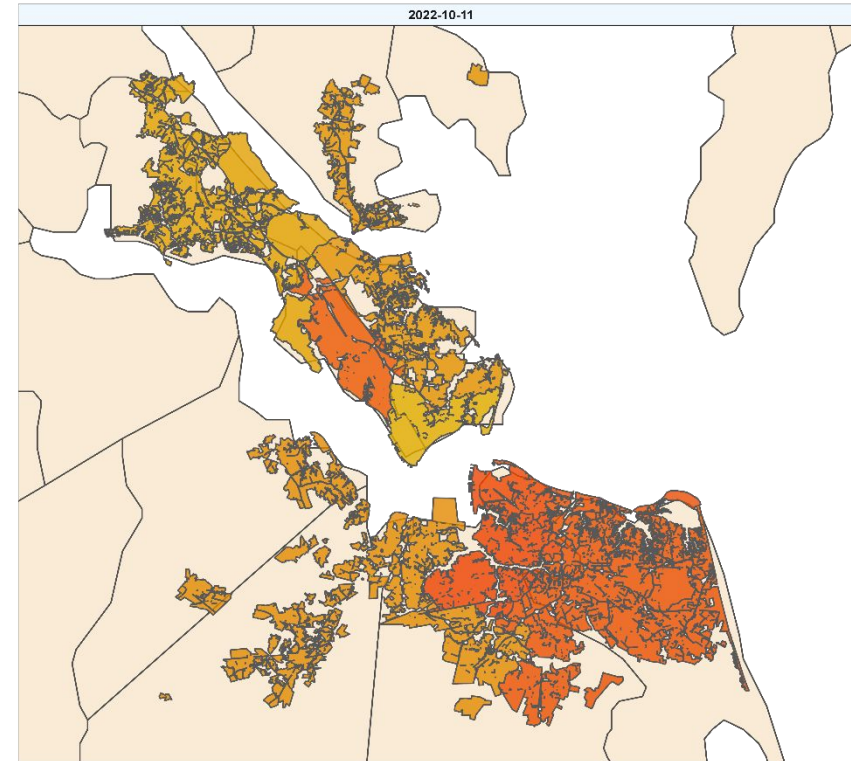
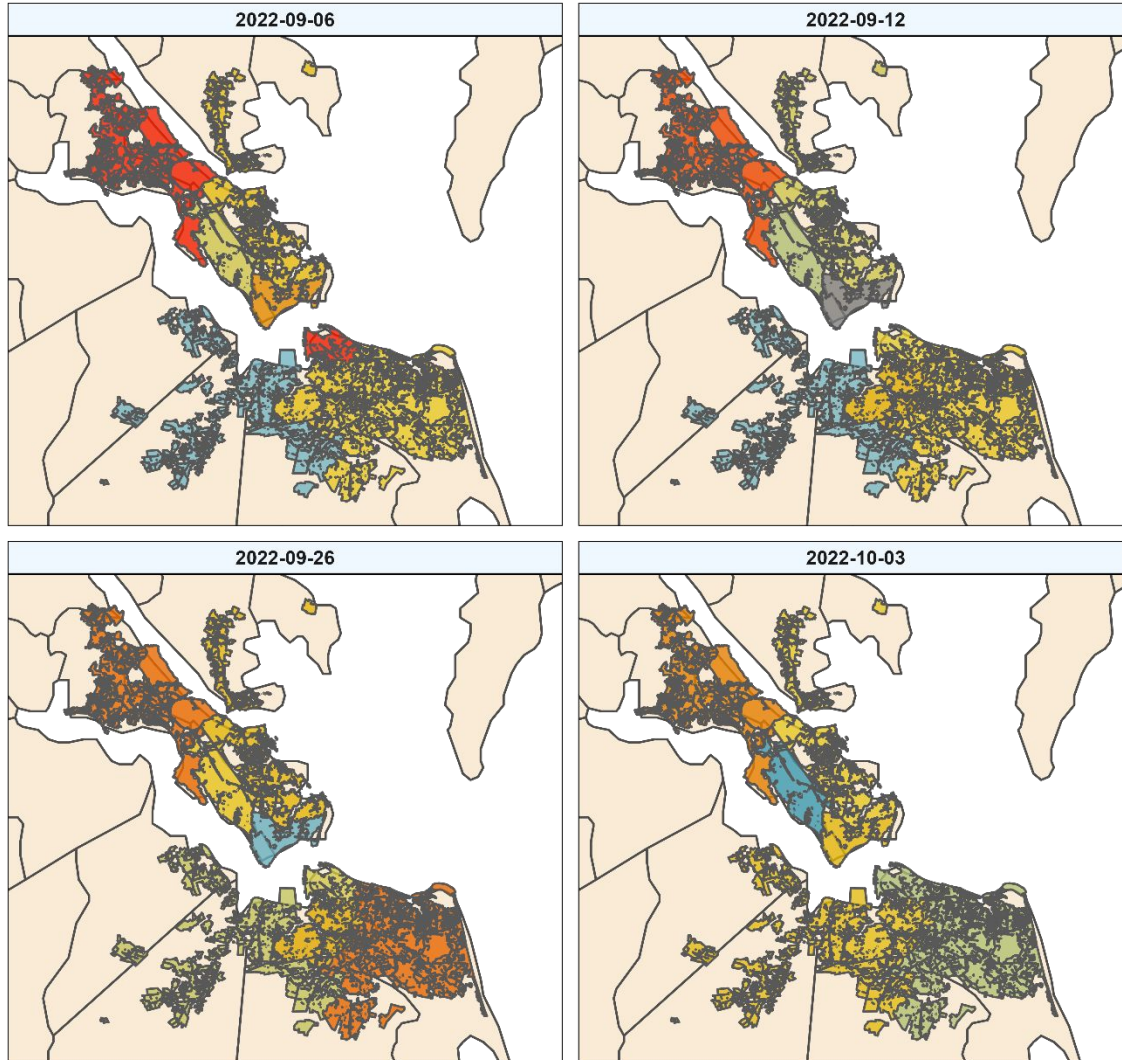


### COVID-19 Hospitalizations and Deaths

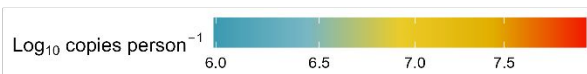
Most Recent 8 Weeks



# SARS-CoV-2 Most Recent 5 Weeks

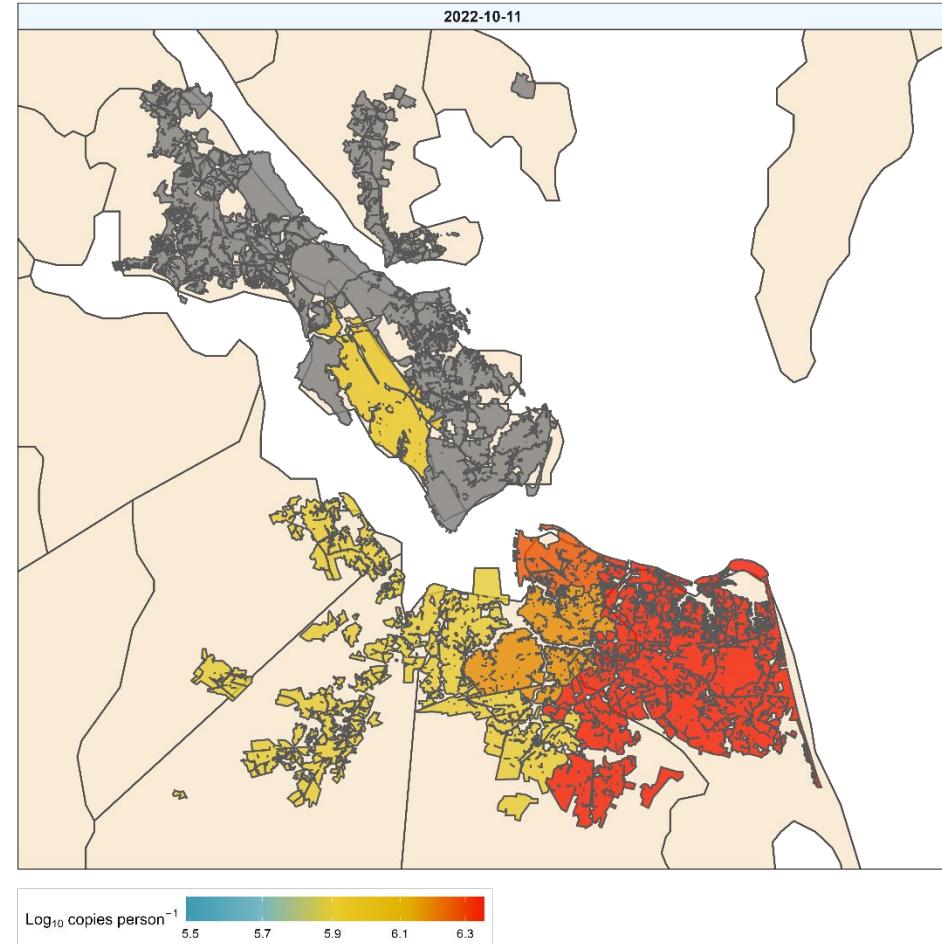
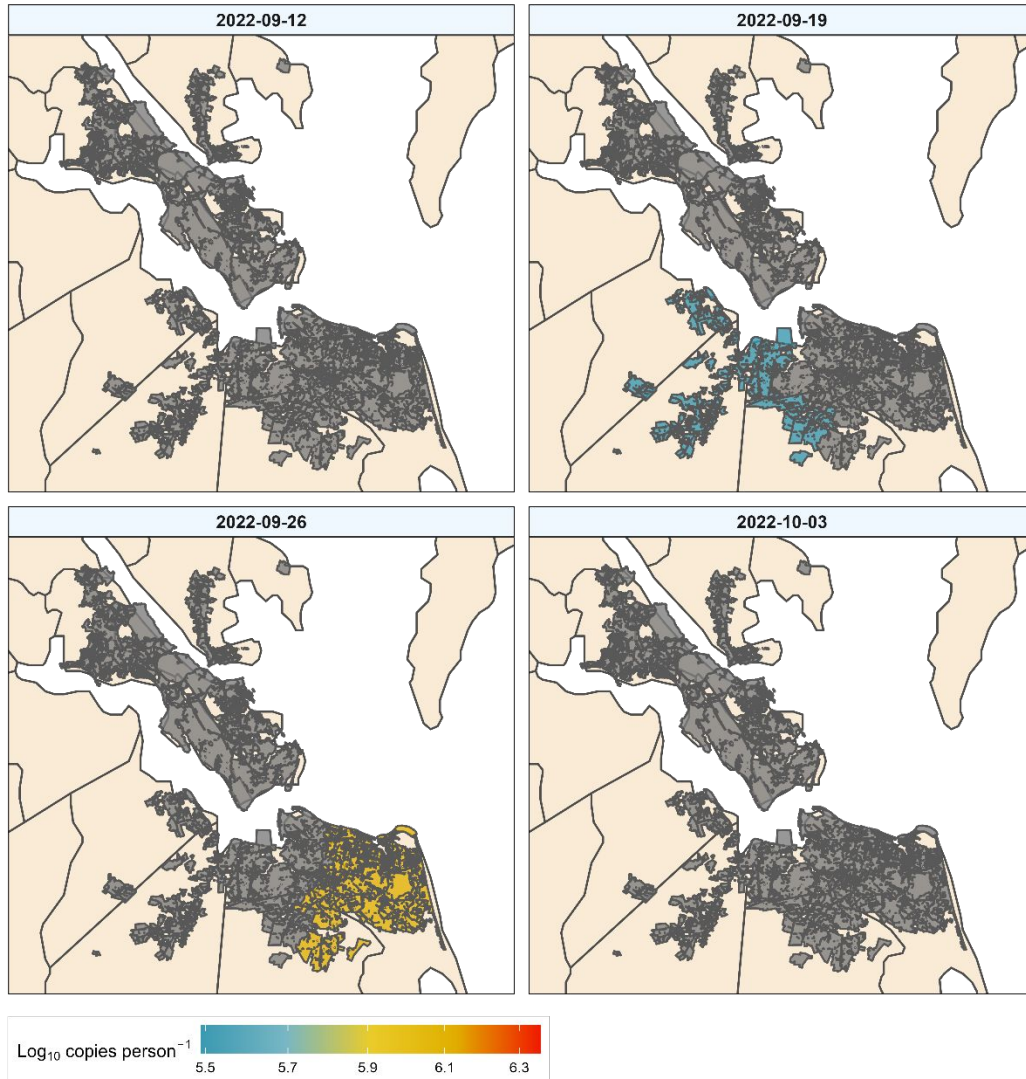


\*\* Note that the scale for this heatmap is now based on the range of the most recent 5 weeks of data.





# Influenza



I) **Title/Test Code:**  
Droplet Digital Polymerase Chain Reaction (ddPCR)

II) **Test Application:**  
Droplet Digital polymerase chain reaction (ddPCR) provides absolute quantification of nucleic acid target sequences for the analysis of recreational waters and wastewater. Applications include source tracking of fecal contamination in recreational waters, wastewater-based epidemiology, tracking viruses and bacteria through wastewater treatment processes, and fate and transport after discharge into receiving waters. Droplet Digital PCR measures absolute quantities by counting fluorescently tagged nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions after amplification on a thermal cycler. Nucleic acid is extracted from sample filters, combined with master mix, containing primers and probes specific to the target, and partitioned into droplets on a droplet generator through water-oil emulsion droplet technology with microfluidics. These samples are partitioned into up to 20,000 droplets and PCR amplification of each droplet is carried out on a thermal cycler. After PCR, droplets are streamed in a droplet reader, which counts the fluorescent positive droplets and calculates target nucleic acid concentration.

#### D) Appendix

#### III) General Safety

In compliance with OSHA regulations for "Occupational Safety and Health Administration, the Water Quality Department and implemented a written program known as the WQ (WQ CHP) which sets forth procedures, equipment, personal protective equipment and work practices that will protect employees and keep employee exposure to hazardous chemicals at or below exposure limits specified in the standard. All personnel must familiarize themselves with the WQ CHP and adhere to the WQ CHP is the protection of the employee.

The toxicity or carcinogenicity of each reagent used in this procedure. Each chemical should be regarded as a hazard and exposure should be as low as reasonably achievable. Safety Data Sheet (SDS) information and an SDS is available in this procedure. It is the responsibility of all personnel with the chemicals they use in the workplace to review the chemical for the first time. Personnel should periodically review chemicals used on a routine basis.

Chemicals must be stored appropriately according to their hazard (e.g., Acids, caustics, flammable, non-flammable) and must be labeled with the identity of their contents and warning.

#### A) DNA Assay Information:

- **16S rRNA:**
  - a) Reference: [Brukner et al. 2015](#)
  - b) Standard: *Escherichia coli* K-12 (ATCC 29425)
  - c) **Primer/Probe:**
    - > **Probe:** 16S\_rRNA\_P1: HEX-CGT ATT ACC GCG GCT GCT GGC AC - [BHQ1 or Iowa Black]
    - > **Forward Primer:** 16S\_rRNA\_F: TCC TAC GGG AGG CAG CAG T
    - > **Reverse Primer:** 16S\_rRNA\_R: GGA CTA CCA GGG TAT CTA ATC CTG TT
  - d) **Annealing Temperature:** 63°C
- **Adenovirus 40/41:**
  - a) Reference: [Jothikumar et al. 2005](#)
  - b) Standard: ATCC Standard: ATCC VR-930D
  - c) **Primer/Probe:**
    - > **Probe:** JTVXP:\_[FAM] CTG GTG CAG TTC GCC CGT GCC A [BHQ]
    - > **Forward Primer:** JTVXF: GGA CGC CTC GGA GTA CCT GAG
    - > **Reverse Primer:** JTVXR: ACI GTG GGG TTT CTG AAC TTG TT
  - d) **Annealing Temperature:** 55°C
- **Adenovirus spp. (total):**
  - a) Reference: [Jothikumar et al. 2005](#)
  - b) Standard: ATCC Standard: ATCC VR-930D
  - c) **Primer/Probe:**
    - > **Probe:** 18923P: FAM-CTG GTG CAG TTC GCC CGT GCC A-BHQ1
    - > **Forward Primer:** 18895F: GGA CGC CTC GGA GTA CCT GAG G
    - > **Reverse Primer:** 18990R: ACN GTG GGG TTT CTG AAC TTG TT
  - d) **Annealing Temperature:** 55°C
- **ARG Assay: intI1:**
  - a) Reference: HRS-D
  - b) Standard: *gBlock* Standard args2: intI1: AAA CCG AGG ATG CGA ACC ACT TCA TCC GGG GTC AGC ACC ACC GGC AAG CGC CGG GAC GGC CGA GGT CTT CCG ATC TCC TGA AGC CAG GGC; CTX-M\_all: GGT GCA GCG AGC ATT CAG GCT GGA CTG CCT GCT TCC TGG GTT GTG GGG GAT AAA ACC GGC AGC GGT GAC TAT GGC ACC ACC AAC GAT ATC GCG GTG ATC TGG CCA AAA GA; CTX-M-32: AAG CGA ACC GAA TCT GTT AAA TCA GCG AGT TGA GAT CAA AAA ATC TGA CTT GGT TAA CTA TAA TCC GAT TGC GGA AAA GCA CGT CGA TGG GAC GAT GTC ACT GGC TGA G

## Faster Assay Incorporation

- Already know the workflow for different types of targets—DNA, RNA, viruses, bacteria, fungi
- ddPCR SOP in place
  - Amend the appendix
- Spend time on
  - In silico testing
  - Instrument validation and optimization

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# Monkeypox Case Study

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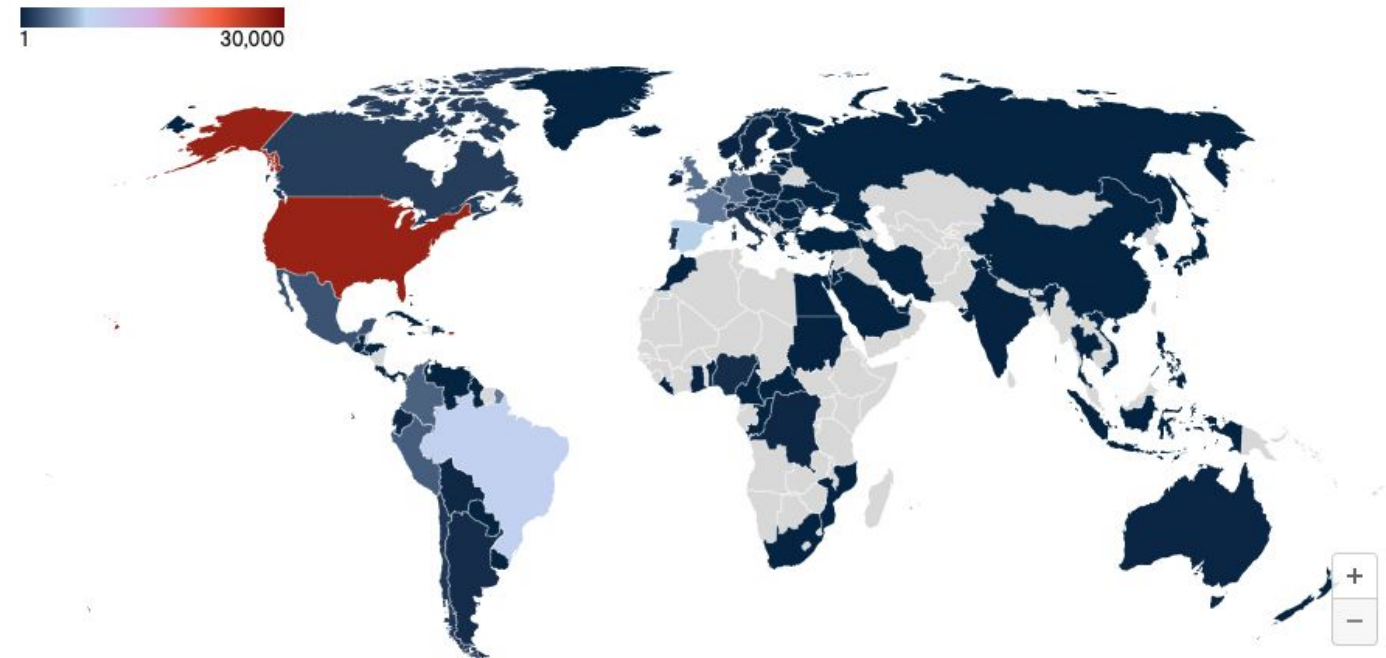
## Timeline:

<https://www.thinkglobalhealth.org/article/monkeypox-timeline>

### Status as of October 21, 2022

- Total Cases Confirmed Globally: **75,345**
- Total Deaths Confirmed Globally: **32**
- Number of Countries with Confirmed Cases: **109**

Confirmed Monkeypox Cases Since January 2022



Last Updated: October 21, 2022

Map: CFR/Isabella Turilli • Source: [World Health Organization](#)

Think Global Health

### In silico:

- Assay choice
  - Or primer/probe design
- Sensitivity
- Specificity

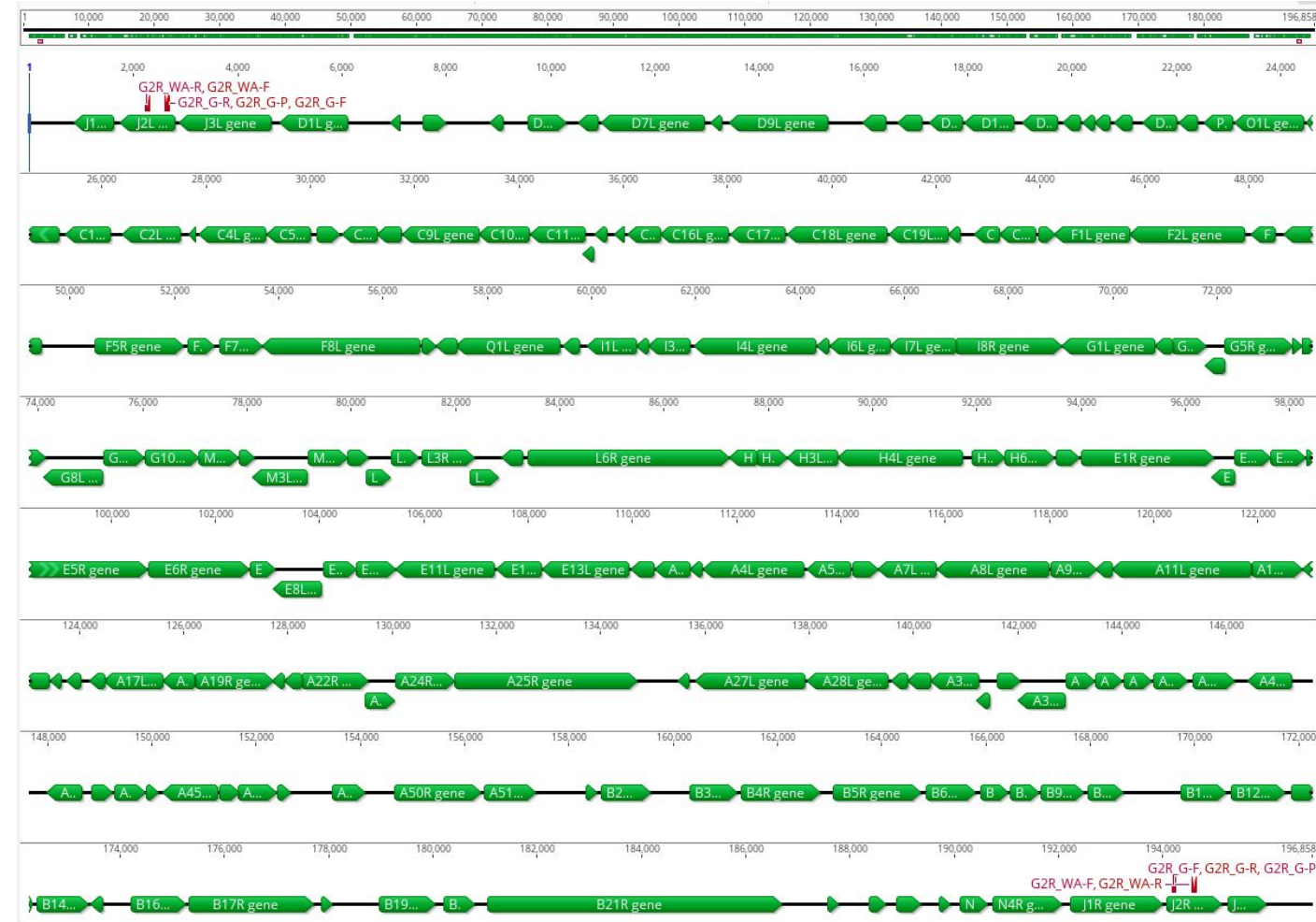
### In the lab (4-8 hours):

- Thermal Gradient
- LOD determination
- Multiplexing potential
- Sensitivity/specificity

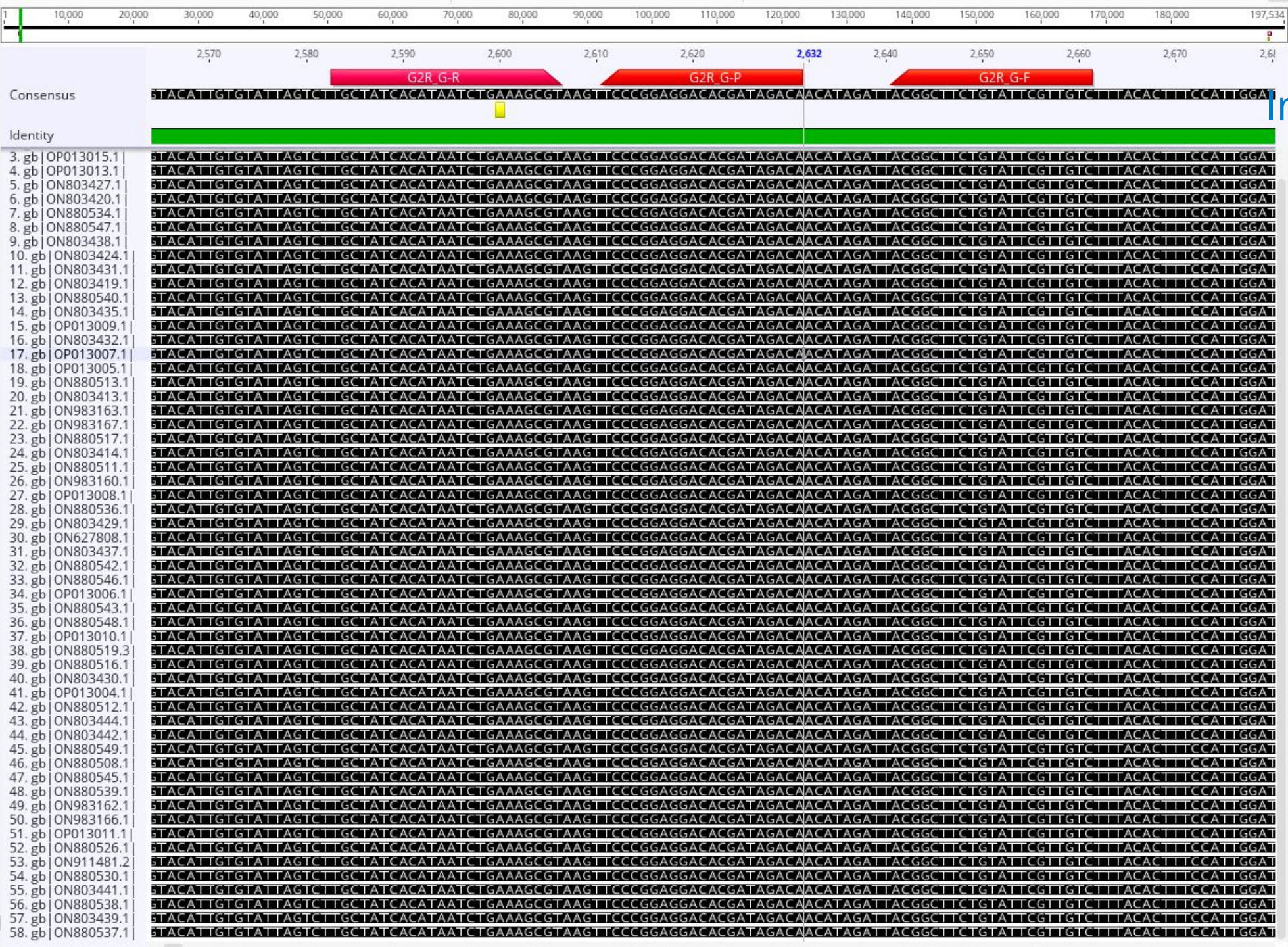
# In silico: Assay Choice

**Table 1. Monkeypox target sequences**

Target	Purpose	Source
E9L-NVAR	CDC Assay, all MPXV	CDC <a href="https://doi.org/10.1016/j.jcv.2006.03.012">https://doi.org/10.1016/j.jcv.2006.03.012</a>
G2R_G	Assay with G2R_G primers and probe: detects all MPXV strains	CDC <a href="https://doi.org/10.1016/j.jviromet.2010.07.012">https://doi.org/10.1016/j.jviromet.2010.07.012</a>
G2R_WA	Assay with G2R_WA primers and probe: detects Western African clade viruses	CDC <a href="https://doi.org/10.1016/j.jviromet.2010.07.012">https://doi.org/10.1016/j.jviromet.2010.07.012</a>
C3L	Assay with C3L primers and probe: detects Congo Basin clade viruses	CDC <a href="https://doi.org/10.1016/j.jviromet.2010.07.012">https://doi.org/10.1016/j.jviromet.2010.07.012</a>
F3L	conserved for specifically detecting MPXV	US Army <a href="https://doi.org/10.1038/labinvest.3700143">https://doi.org/10.1038/labinvest.3700143</a>
N3R	conserved for specifically detecting MPXV	US Army <a href="https://doi.org/10.1038/labinvest.3700143">https://doi.org/10.1038/labinvest.3700143</a>
E9L-OPX3	CDC Assay, all MPXV	CDC <a href="https://doi.org/10.4269/ajtmh.2010.09-0716">https://doi.org/10.4269/ajtmh.2010.09-0716</a>
B6R	CDC Assay; selective for MPXV	CDC <a href="https://doi.org/10.1016/j.jcv.2006.03.012">https://doi.org/10.1016/j.jcv.2006.03.012</a>
B2R	Poxin gene associated with toxin	<a href="https://doi.org/10.1038/s41586-019-0928-6">https://doi.org/10.1038/s41586-019-0928-6</a>







# In silico: Sensitivity





# In silico: Primer Blast

>NC\_003310.1 Monkeypox virus, complete genome

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 2226 ..... 2250

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 2312 ..... 2293
```

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 194633 ..... 194609

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 194547 ..... 194566
```

>NC\_008291.1 Taterapox virus, complete genome

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 2235 ..... 2259

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 2321 .....A..... 2302
```

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 195816 ..... 195792

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 195730 .....A..... 195749
```

>NC\_066642.1 Horsepox virus, complete genome

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 2162 ..... 2186

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 2248 .....G. 2229
```

product length = 87

>NC\_003391.1 Camelpox virus, complete genome

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 3716 ..... 3740

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 3802 ....G.....G. 3783
```

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 202004 ..... 201980

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 201918 ....G.....G. 201937
```

>NC\_006998.1 Vaccinia virus, complete genome

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 4952 .....A..... 4976

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 5038 .....G. 5019
```

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 189760 .....A..... 189736

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 189674 .....G. 189693
```

>NC\_001611.1 Variola virus, complete genome

```
product length = 87
Forward primer 1 TGGATACAGGTTAATTTCCACATCG 25
Template 183112 .....TG..... 183088

Reverse primer 1 TCTCACACCGTCTCTTCCAC 20
Template 183026 .....G. 183045
```



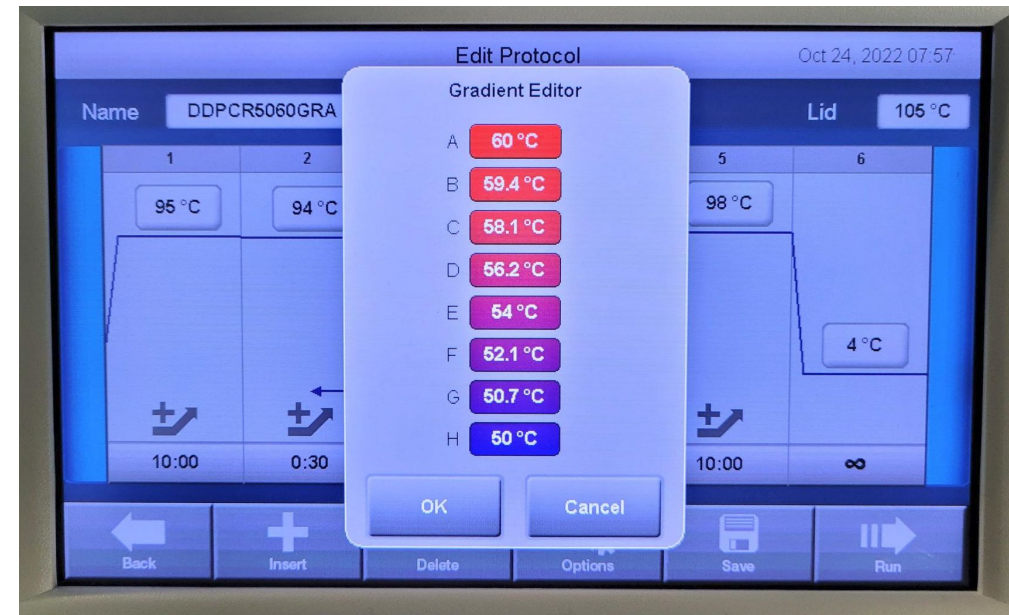
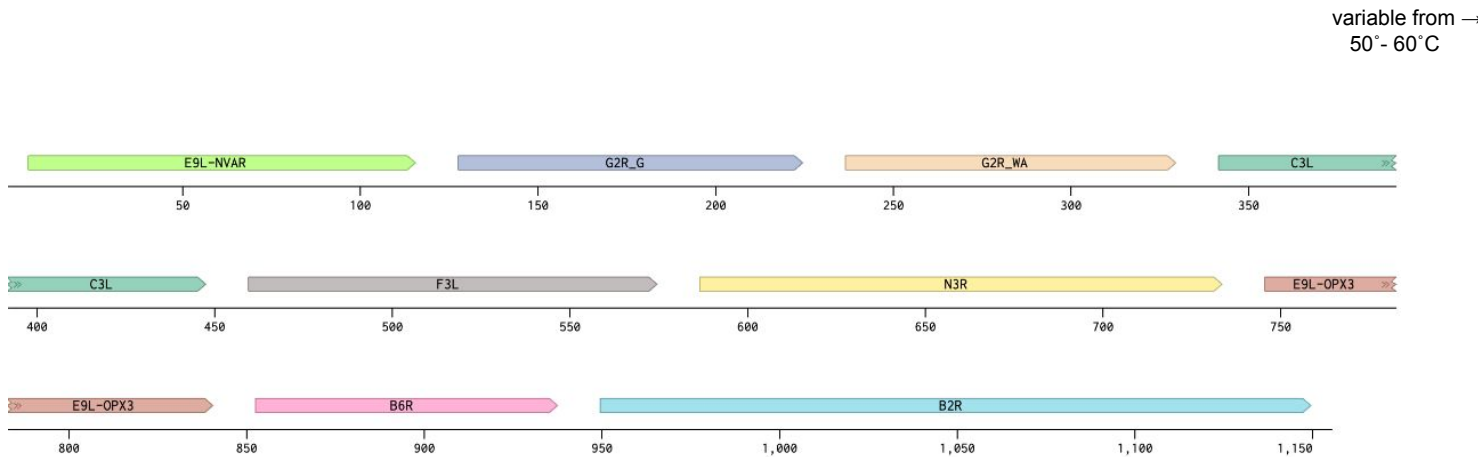


# Lab Optimization and Validation

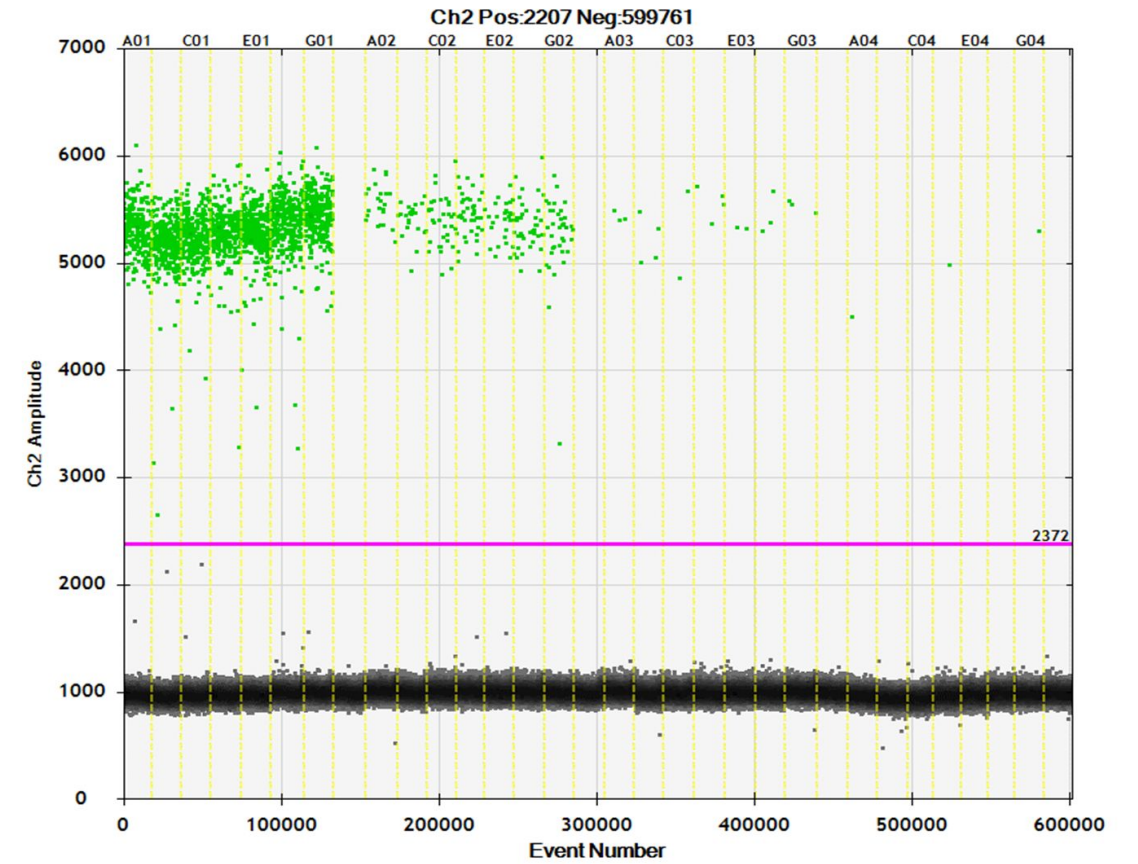
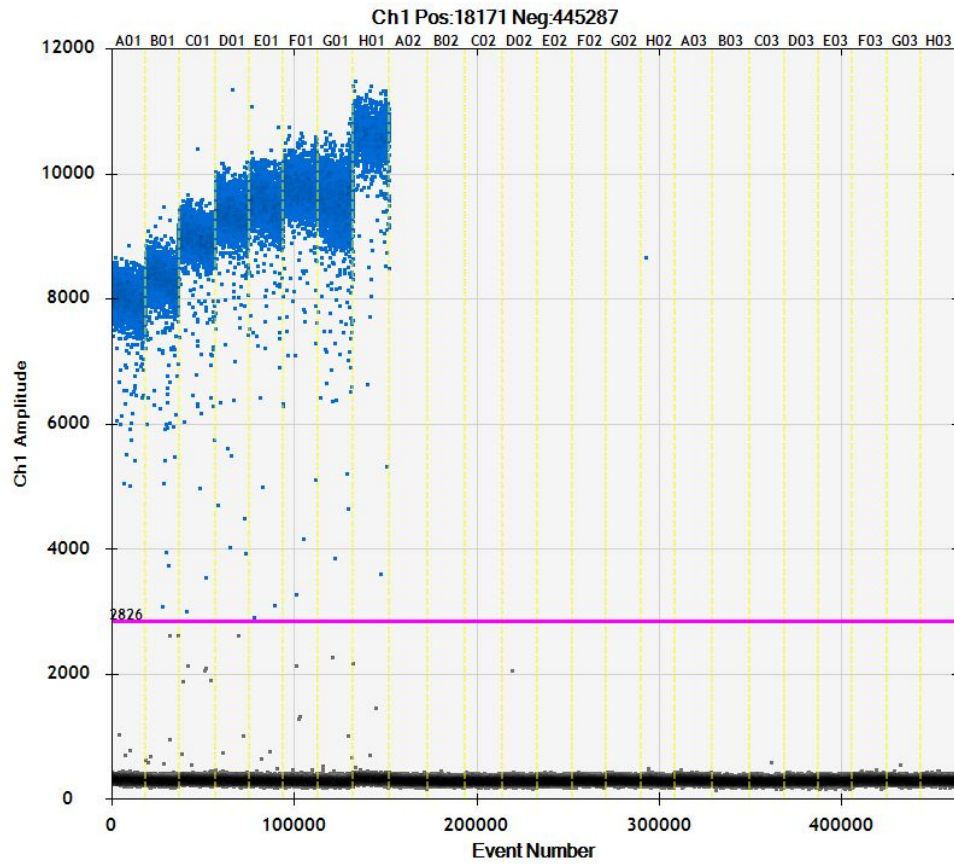
Table 2. Cycling conditions for Bio-Rad's C1000 Touch Thermal Cycler.\*

Cycling Step	Temperature, °C	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	10 min	2°C/sec	1
Denaturation	94	30 sec		40
Annealing/extension	60	1 min		40
Enzyme deactivation	98	10 min		1
Hold (optional)	4	Infinite		1

\* Use a heated lid set to 105°C and set the sample volume to 40 µl.

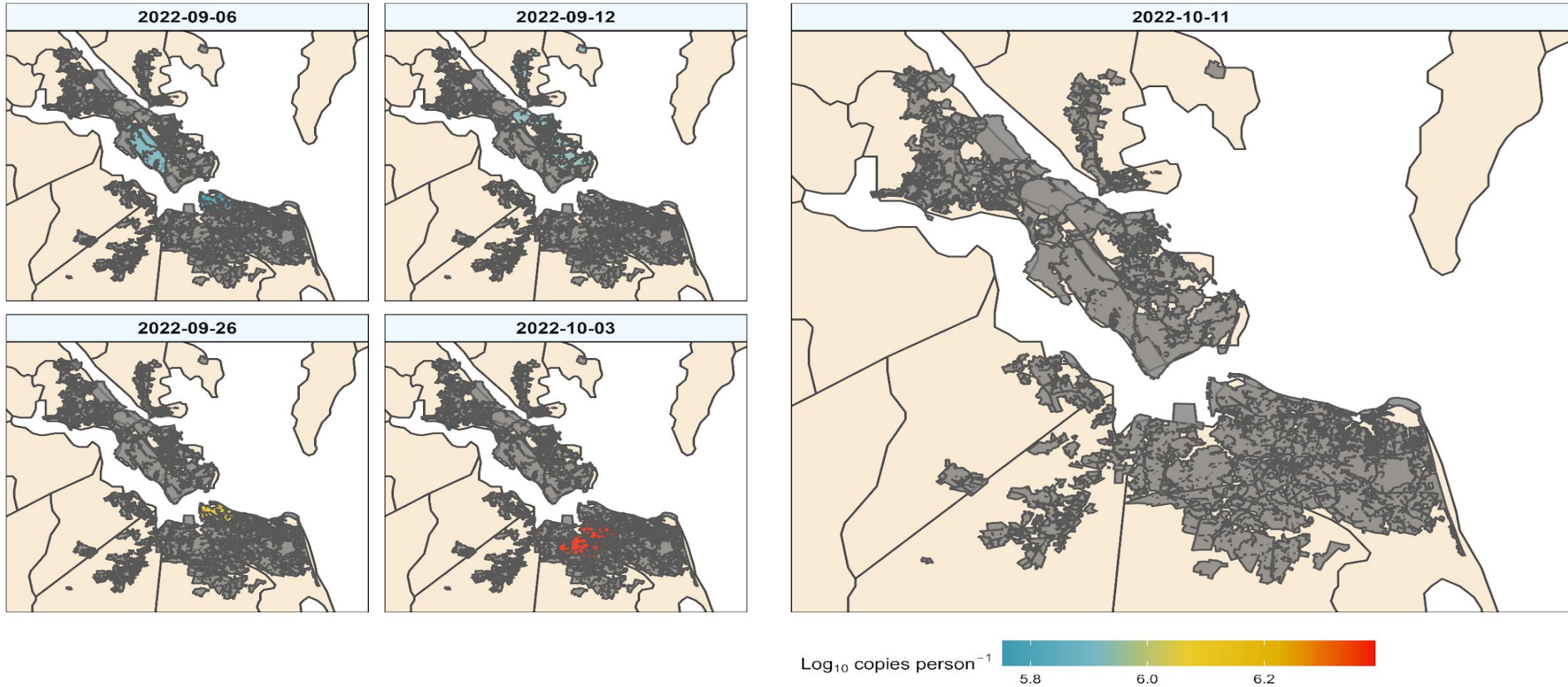


# Monkeypox First Run





# Monkeypox



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Questions?

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# Questions and Open Discussion

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thank  
you!

See you Soon!

Send inquiries / topics to:  
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