



# Wastewater-based genomic epidemiology for SARS-CoV-2 surveillance in South Africa

**23 February 2024**

Sample collection dates up to 16 February 2024  
(Epidemiological week 7)

C. Sankar\*, P. Ntuli\*, F. Els, N.S Msomi, N.L Ndlovu, V. Mabasa, E. Phalane, M.E Macheke, S. Gwala, N. Singh, T. Mangena, M Motloug, L. Monametsi, L.P Rabotapi, S. Maposa, M. Yousif#, K. McCarthy#

\*Joint first authors

#Joint last authors

Centre for Vaccines and Immunology,  
National Institute for Communicable Diseases,  
A division of the National Health Laboratory Service



**NATIONAL INSTITUTE FOR  
COMMUNICABLE DISEASES**

Division of the National Health Laboratory Service

# Summary: SARS-CoV-2 transmission and genomics based on evaluation of wastewater at sentinel sites across RSA

## Wastewater levels

### Epidemiological weeks 48 (2023) – 7 (2024)

- From weeks 48-7 the cumulative SARS-CoV-2 levels measured at wastewater treatment works (WWTW) has remained around one log genome copies/ml of wastewater. This has followed on from the increases observed in weeks 31-41, when the cumulative SARS-CoV-2 levels in wastewater at sentinel sites in South Africa showed increases to levels above 2 log (100) genome copies/ml of wastewater, up from below one log copy/ml in epidemiological week 22 (first week in June 2023). From week 45, levels measured below 2 log genome copies/ml of wastewater.
- In weeks 48-52 increases and/or higher levels have been seen in Gauteng (Goudkoppies WWTW). In weeks 1-6 lower levels were observed nationally, with an increase seen in week 7.
- Correlation with syndromic surveillance for influenza-like illness (ILI) and severe acute respiratory infection findings (SARI) is required to determine the clinical and public health significance of ongoing transmission.  
<https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-respiratory-pathogens-surveillance-report-week/>

## Wastewater genomics

### Epidemiological weeks 42-46

- No new sequencing results available for Epi week 47
- Omicron lineage **XBB.1.41.1** followed by **BA.2.86**, **JB.2**, **JB.2.1** and XBB sub-lineages were the dominant lineages circulating in wastewater samples between October and November 2023
- In clinical samples, **BA.2.86** was also the dominant lineage circulating throughout October 2023, followed by **XBB.1.5**, **XBB.1.16** and **XBB.1.5.81**.
- The Omicron lineage **BA.2.86** is circulating in KwaZulu-Natal in eThekweni (in the catchments of Northern and Central WWTWs), and in Gauteng, in the City of Johannesburg (in catchments of Northern and Goudkoppies WWTWs), in Ekurhuleni (in the catchments of Olifantsfontein, Vlakplaats, and Hartebeesfontein WWTWs), and the City of Tshwane (in the catchment of Daspoort WWTP). It is also circulating in Eastern Cape in Buffalo City (in the catchment of Mdantsane WWTW ), in Western Cape, in the City of Cape Town (in the catchment of Borches Quarry WWTW), and Free State, in Mangaung (catchments Bloemspruit and Sterkwater WWTWs).

**Interpretation:** Ongoing transmission of SARS-CoV-2 due to Omicron lineages including the new lineage BA.2.86.

# Wastewater-based Epidemiology for COVID-19

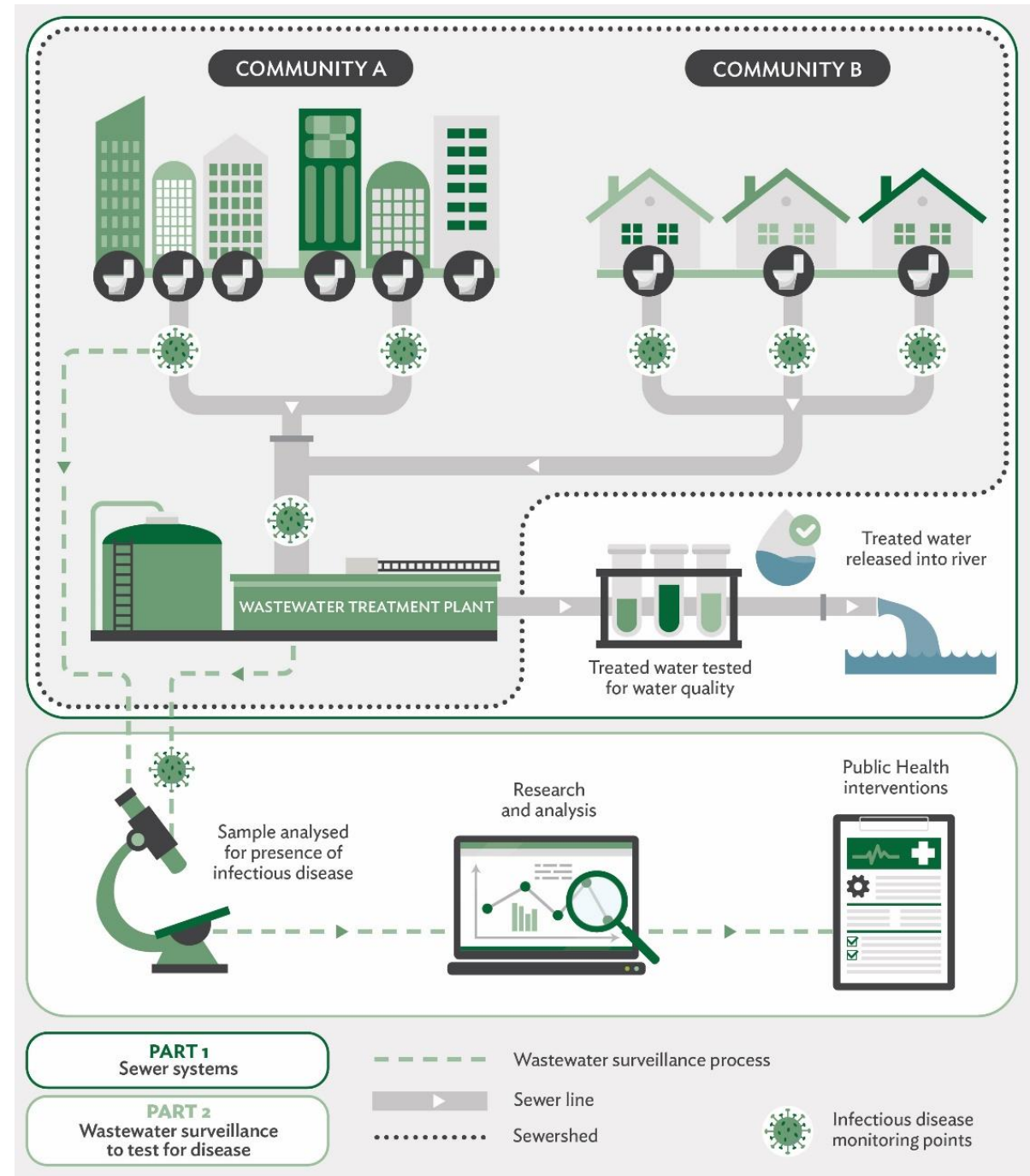
## How is wastewater tested for SARS-CoV-2?

For a full description of this process, see the photo essay developed in collaboration with the Gauteng City Region Observatory <https://www.gcro.ac.za/outputs/photo-essays/detail/photo-essay-sewersheds-what-can-wastewater-tell-us-about-community-health/>

For a technical description and analysis of wastewater levels and results see

<https://pubmed.ncbi.nlm.nih.gov/37506905/>  
<https://www.medrxiv.org/content/10.1101/2022.12.15.22283506v1> (accepted by Nature Communications, publication pending)

SARS-CoV-2 is not transmitted by faeco-oral route. Wastewater with SARS-CoV-2 is not infectious



# Wastewater-based Epidemiology for COVID-19

## What does wastewater testing for SARS-CoV-2 mean?

Left vertical axis:

Number of lab confirmed cases in *the metro or district where the water treatment plant is located*

Bars:

Number of lab confirmed clinical cases in specimens submitted to NICD from persons in the metro/subdistrict where the plant is located

Horizontal axis:

Epidemiological weeks from 2021 to 2023

Coloured lines:

Changes in wastewater SARS-CoV-2 results over time for different treatment facilities

Coloured squares:

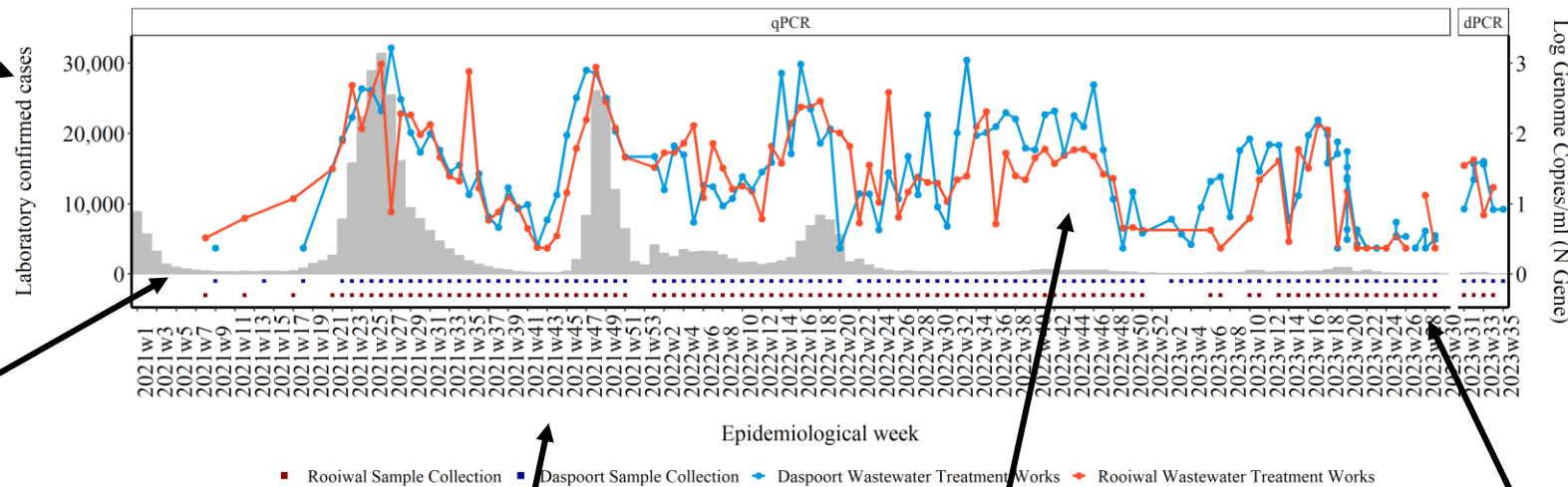
Epi weeks during which samples were collected

Facets:

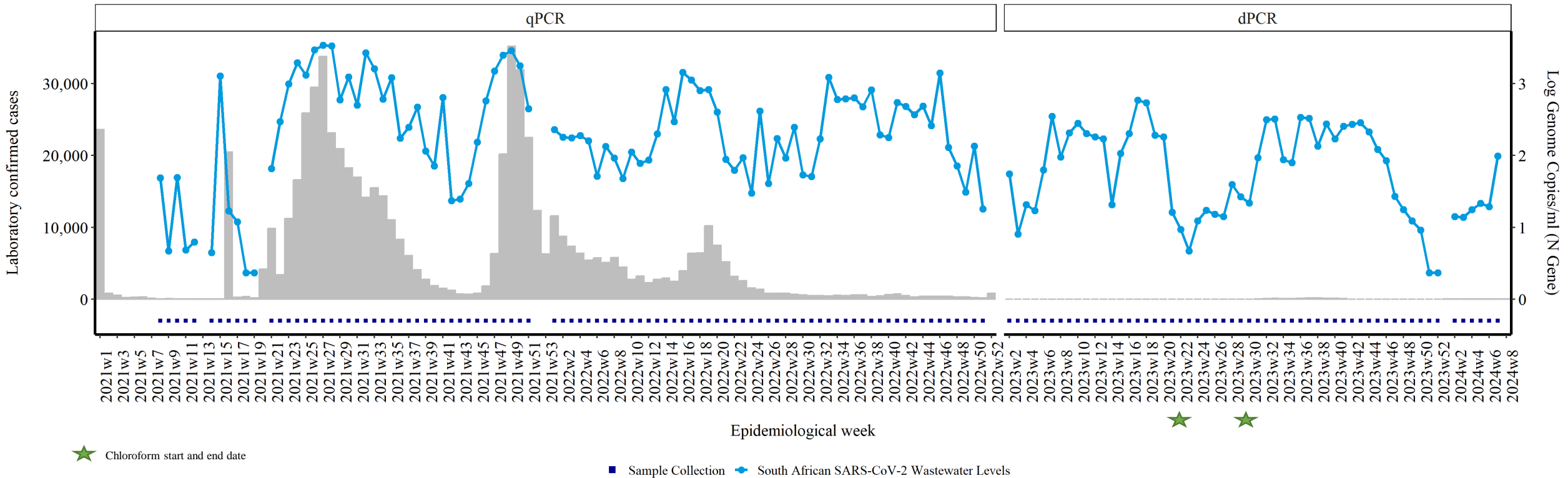
Indicates the platform used to test samples

Right vertical axis:

Log (ie 10 to the power x) copies of SARS-CoV-2 genome per ml of wastewater. So  $\log 2=10^2=100$  copies per millilitre,  $\log 3=10^3=1000$  copies per millilitre



# South Africa at a glance: Summed total of clinical and genome copies

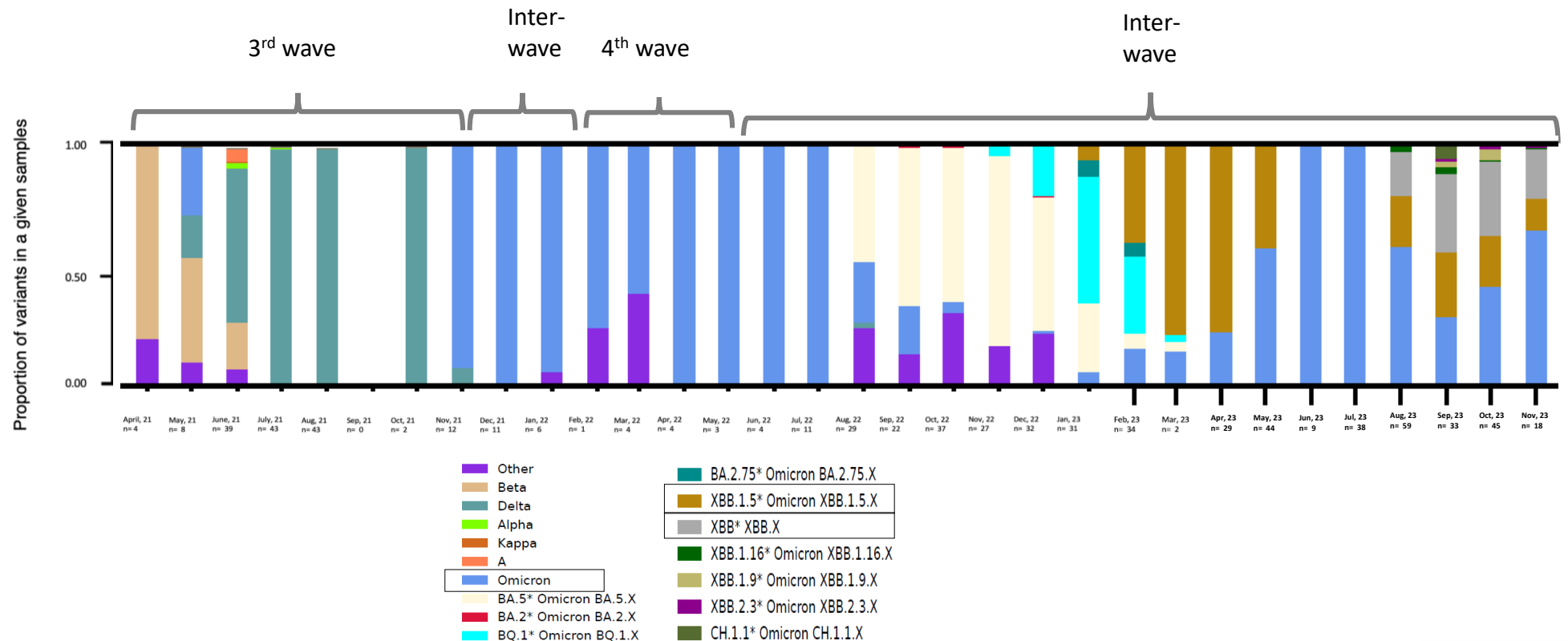


Changes in levels of SARS-Cov-2 (line graph) in in-flowing untreated wastewater from plants tested by NICD, compared with laboratory-confirmed cases from Tshwane, Johannesburg, Ekurhuleni, eThekweni, Mangaung, Nelson Mandela, Buffalo City, and City of Cape Town (grey bars), by epidemiological week, 2021-2023.

# South Africa at a glance: Circulating variants as determined by Freyja deconvolution of sequence data

- SARS-CoV-2 variants in wastewater as determined by the 'Freyja' tool (Scripps Institute)
  - Allows determination of variants in each wastewater sample
- Results from sequencing data ending in epi week 46 (17 November 2023)
  - Omicron lineages XBB.1.5 and XBB\* were circulating from October to November, with an Omicron dominance in November.

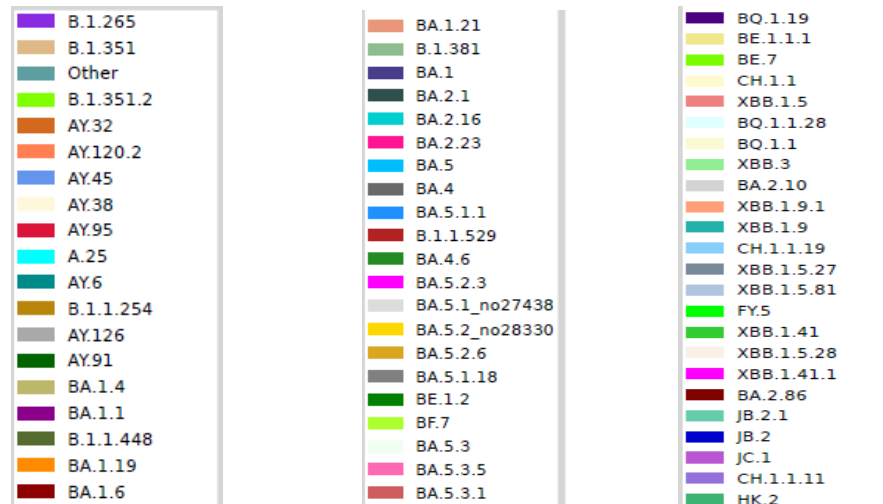
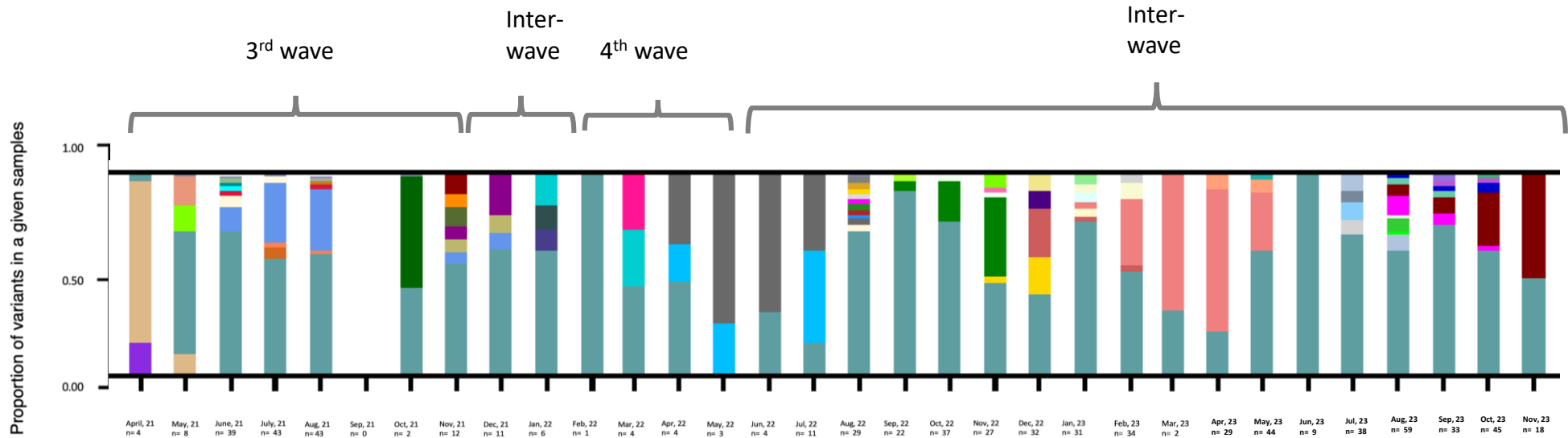
Inferred variants in wastewater samples from South African wastewater treatment plants by month, between April 2021- November 2023



# South Africa at a glance: Circulating lineages as determined by Freyja deconvolution of sequence data

- Results from sequencing data ending in epi week 46 (17 November 2023)

- Omicron lineages XBB.1.41.1 and BA.2.86 were circulating from October to November with BA.2.86 dominance in November.
- Omicron XBB sub-lineages were in circulation throughout October.
- The predominant lineage circulating in clinical samples in the recent week is BA.2.86.



Lineages detected by Freyja in weeks 40-46:

XBB.1.41.1  
JB.2  
JB.2.1  
JC.1  
BA.2.86

# What mutations in the spike protein tell us about the circulating lineages

## Summary:

Summary of lineages associated with most common mutations associated

## Epi Week:

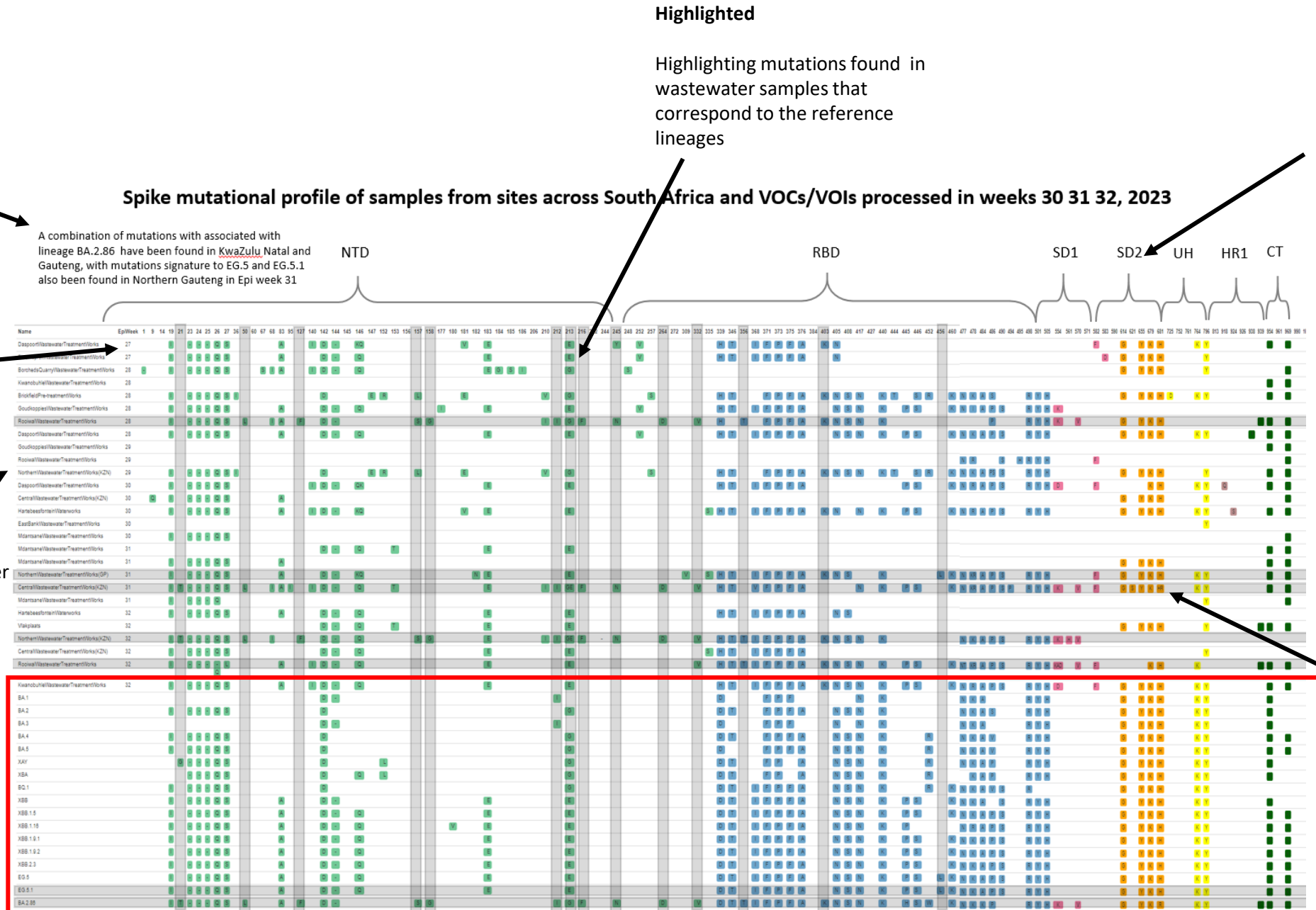
Epidemiological week during which samples were collected

## Site Names:

Sites from which wastewater samples were collected

## Reference lineages:

Reference lineages with signature mutations with which wastewater samples are compared for lineage determination









# Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 42 - 46, 2023

A combination of mutations associated with lineage BA.2.86 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 42-46.



# Amino acid mutations and frequency – Spike protein

XBB\* is a recombinant of BA.2.10.1 and BA.2.75 that is characterised by one or more of the following mutations in the spike protein: V83A, Y144-, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, V445P, G446S, N460K, F486S, F490S

List of variants and sub-lineages of interest and concern  
<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants>

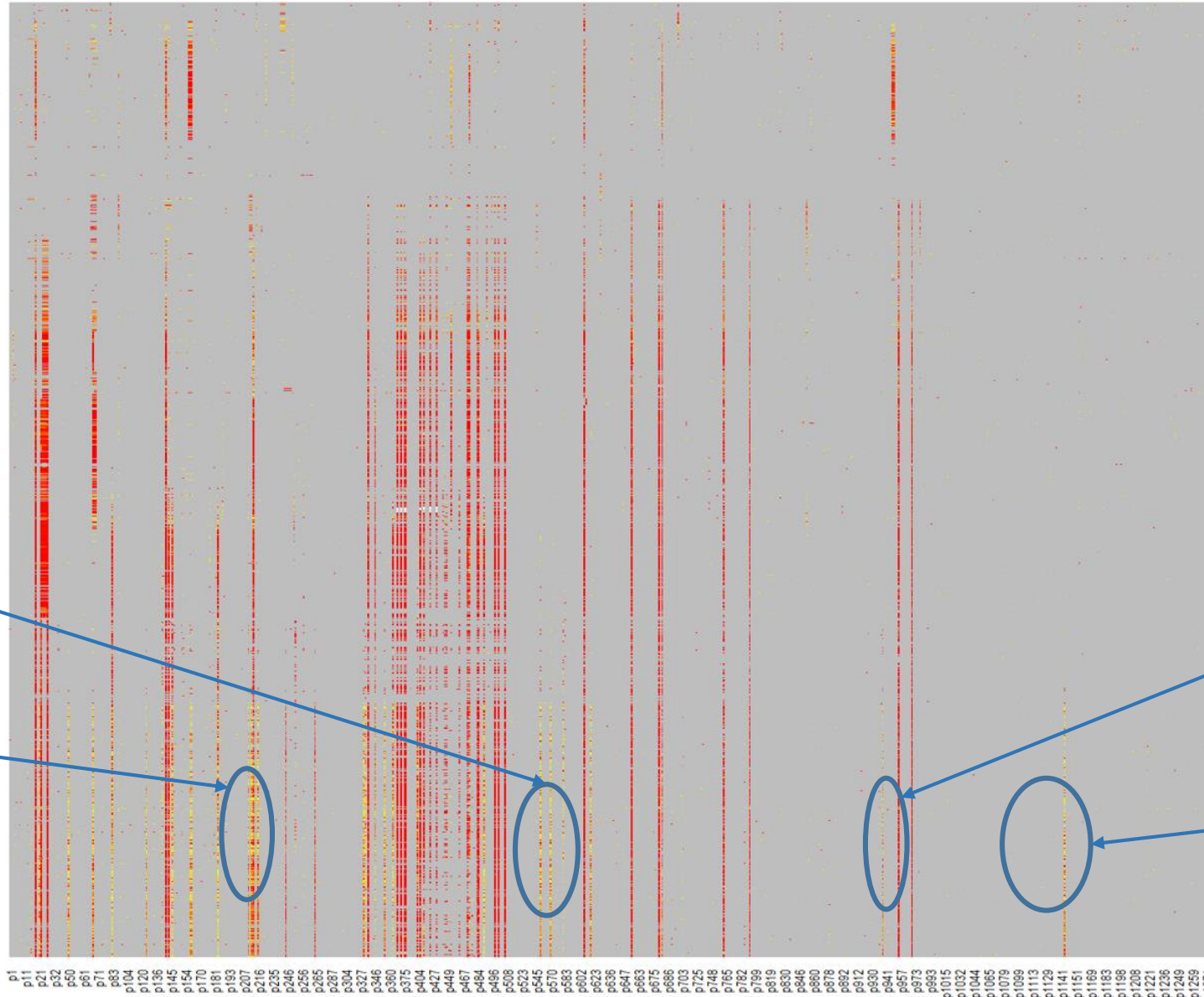
**BA.2.86** is a highly mutated sub-lineage of BA.2, recently circulating in Denmark, Israel and the United States of America and is characterised by one or more of the following mutations in the spike protein: R21T, S50L, H69-, V70-, V127F, F157S, R158G, N211-, L212I, V213G, L216F, H245N, A264D, I332V, K356T, R403K, V445H, N450D, L452W, N481K, V483-, E484K, E554K, A570V, P621S, 1670V, P681R, S939F, P1143L, Ins16:MPLF\*

E554K  
 Mutations in spike protein associated with BA.2.86

V213E,  
 R346T  
 Mutations in spike protein associated with XBB\* sub-lineages

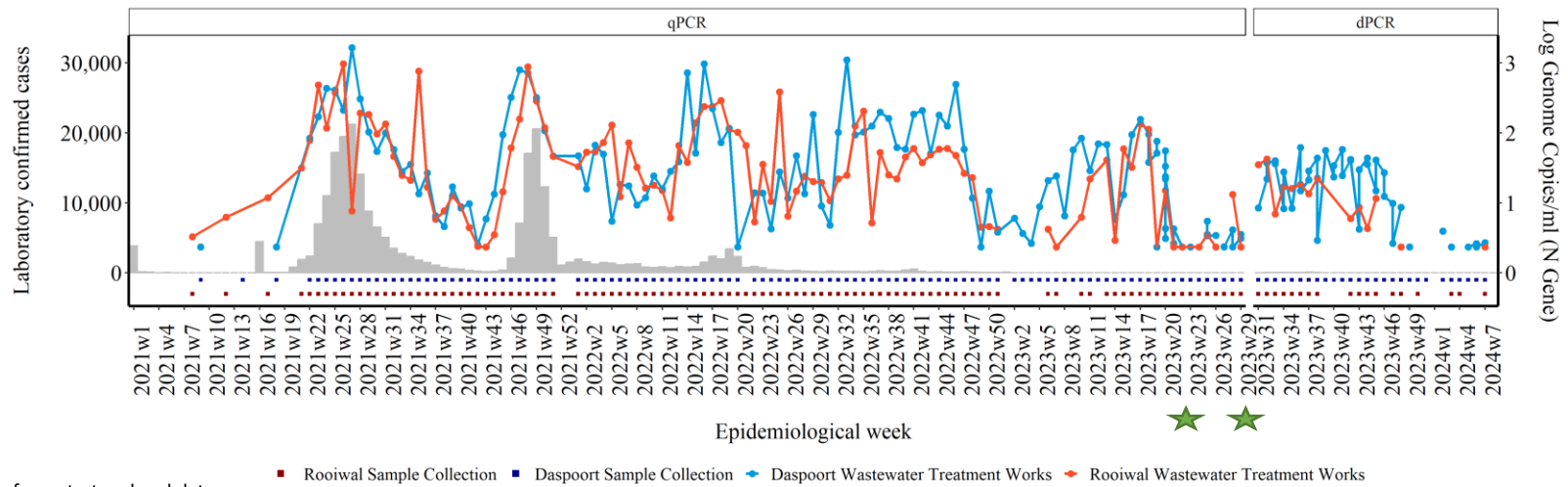
S939F  
 Spike protein mutation associated with the BA.2.86 lineage

P1143L  
 Spike protein mutation associated with the BA.2.86 lineage



Heatmap showing patterns of emerging mutations in the spike region of SARS-CoV-2, collected from April, 2021 - November, 2023. Mutations appearing in yellow have a low read frequency, those appearing in orange have a medium read frequency and those appearing in red have a high read frequency. Mutations are included and updated weekly.

# Gauteng - Tshwane



★ Chloroform start and end date

## SARS-CoV-2 levels and Genomic Results in Epi week 7:

- SARS-CoV-2 levels in Daspoort WWTW decreased to low in Epi week 4 and remain low in Epi week 7.
- SARS-CoV-2 levels in Rooiwal WWTW have decreased in Epi week 47. Levels remain low in Epi week 7.

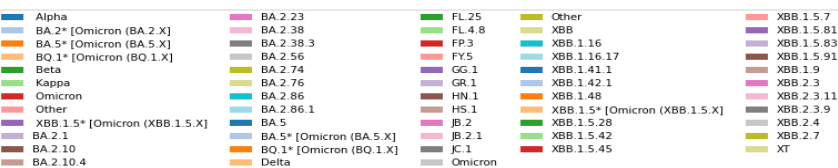
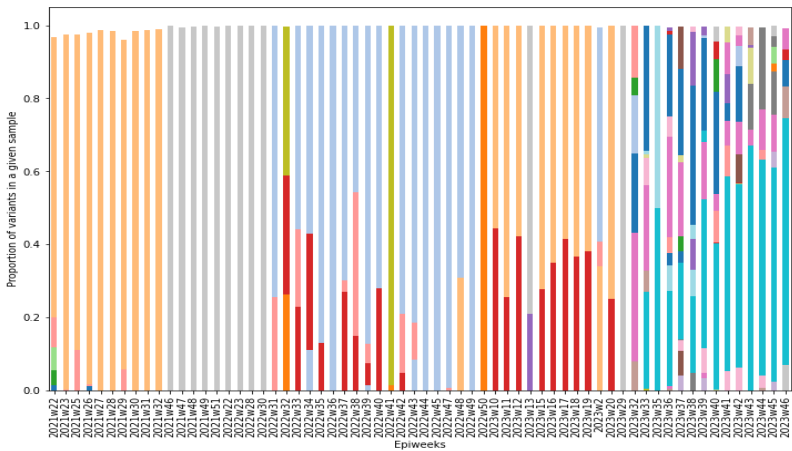
**\* Sequencing data ending in Epi week 46 in Daspoort and 45 in Rooiwal.**

- Omicron lineages HS.1, XBB.1.41, XBB.2.3, BA.2.56 and XBB.1.5.45, were circulating in Daspoort during Epi week 46, with BA.2.86 dominating.
- BA.2.86 was also the dominant lineage detected in Rooiwal during Epi week 45. Other lineages including XBB.1.41, XBB.1.5.28 and XBB.1.42.1 were also in circulation.

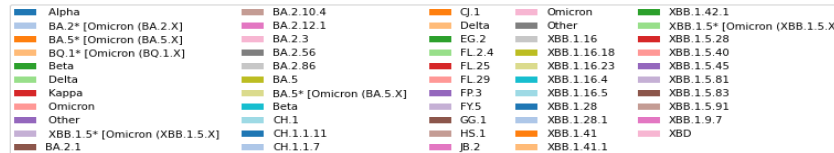
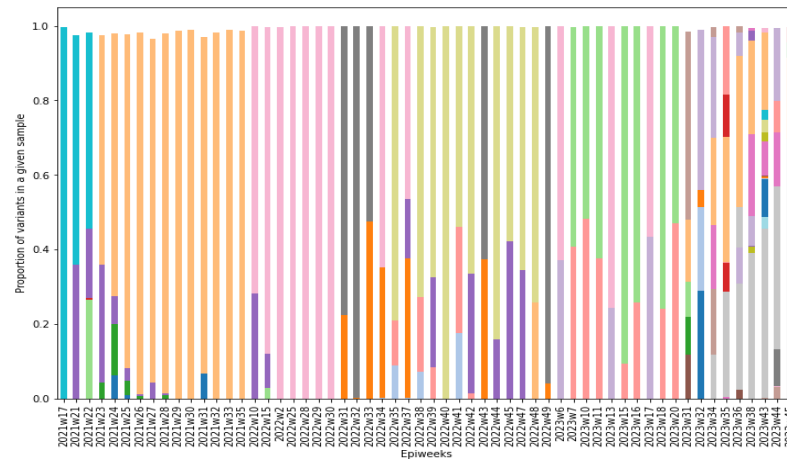
## SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Daspoort and Rooiwal.

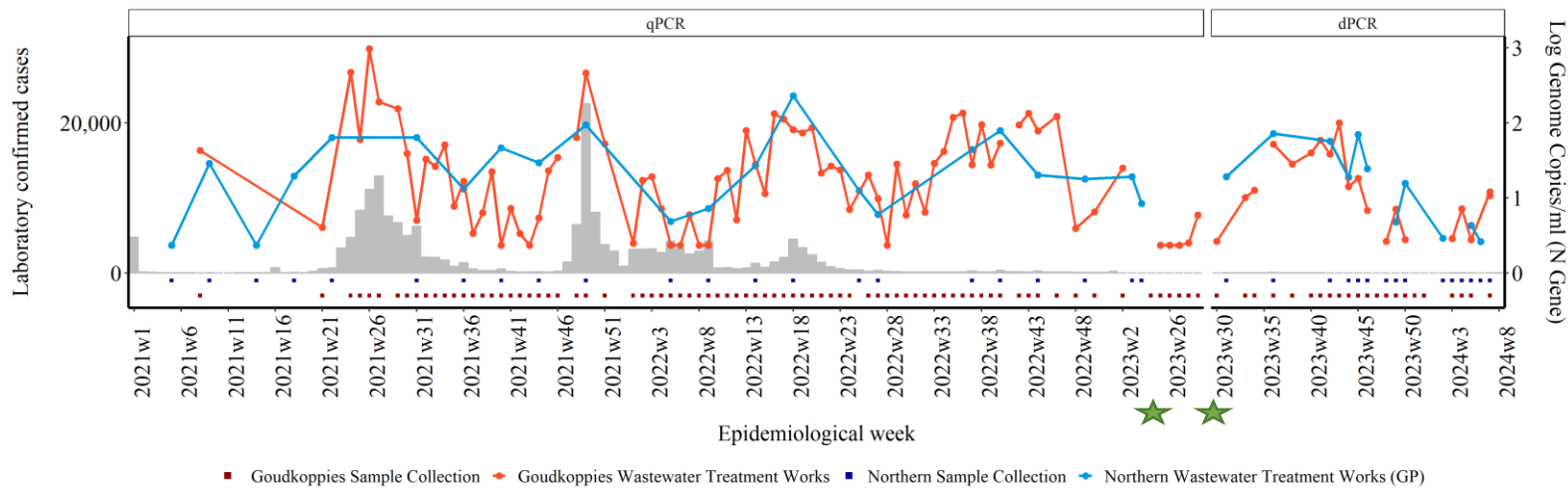
## Daspoort Wastewater Treatment Works



## Rooiwal Wastewater Treatment Works



# Gauteng - Johannesburg



★ Chloroform start and end date

## SARS-CoV-2 levels and Genomic Results in Epi week 7:

- As of Epi week 5, SARS-CoV-2 levels in Goudkoppies WWTW have decreased from levels in week 4. Levels increased from Epi week 5 to Epi week 7, but levels remain low.
- In Northern WWTW, SARS-CoV-2 levels increased slightly in week 5 from levels seen in week 2, and decreased in week 6 as levels remain low. No new results for Epi week 7 are available.

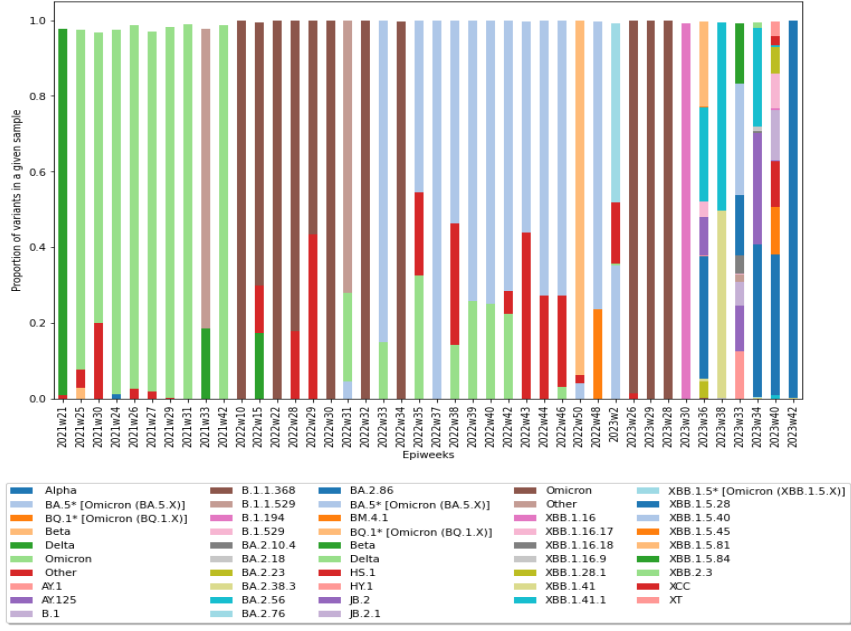
**\* Sequencing data ending in Epi week 42 in Goudkoppies and 45 in Northern.**

- During Epi week 42, Omicron lineage BA.2.86 was dominating in Goudkoppies.
- Omicron lineage BA.2.86 was also dominating in Northern Gauteng. Other lineages in circulation included: XBB.1.41.1, XBB.1.5.45, GA.41.1, BA.2.86.1, JB.2, BA.2.43, EG.5.1, and BA.2.16, during Epi week 45.

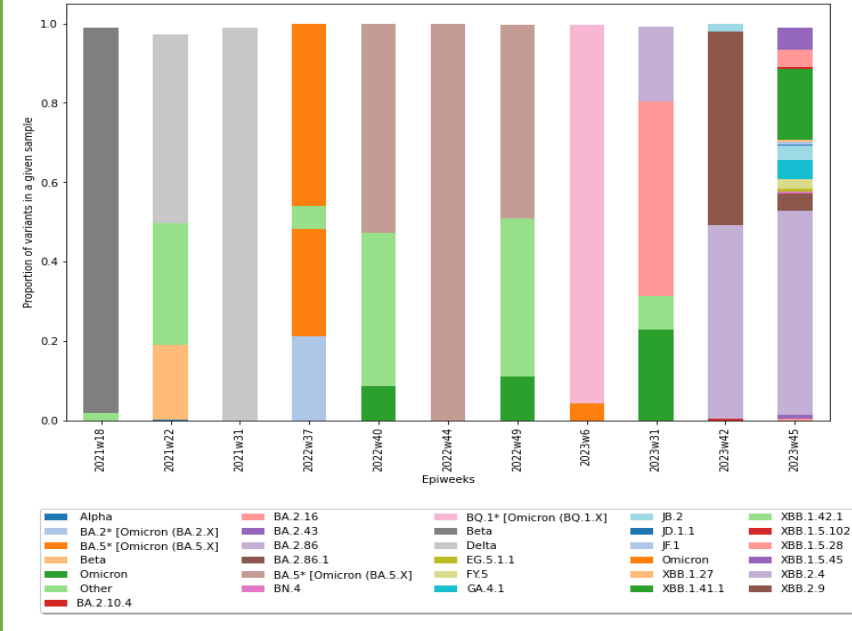
## SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in Goudkoppies.
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in Northern Johannesburg.

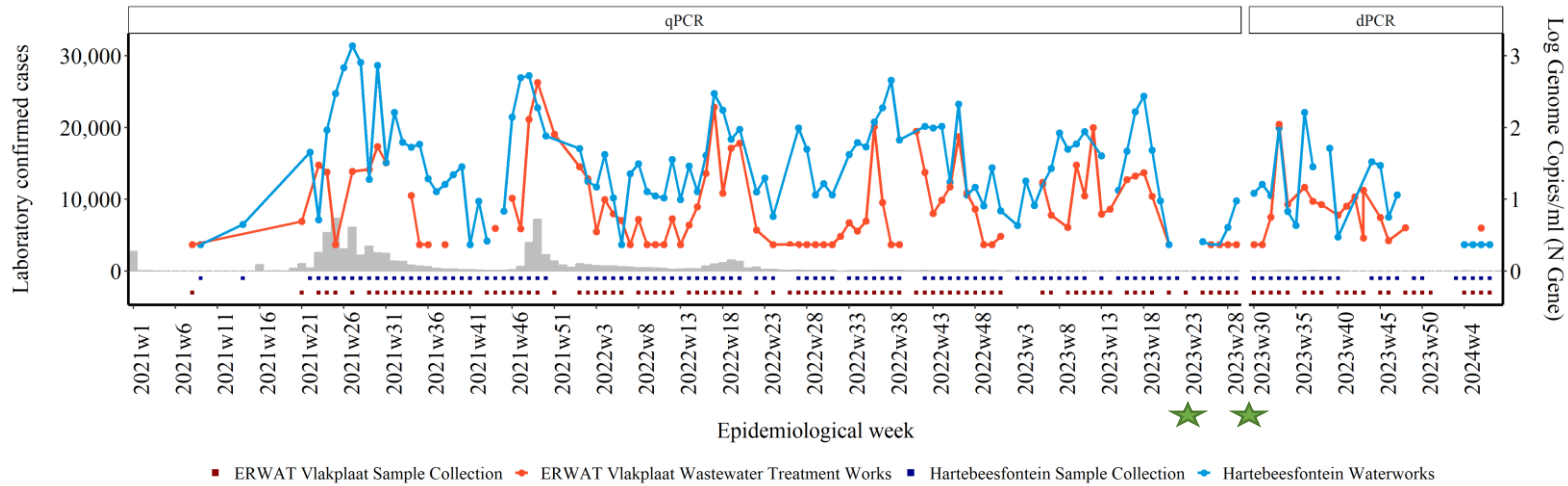
## Goudkoppies Wastewater Treatment Works



## Northern Wastewater Treatment Works



# Gauteng - Ekurhuleni



★ Chloroform start and end date

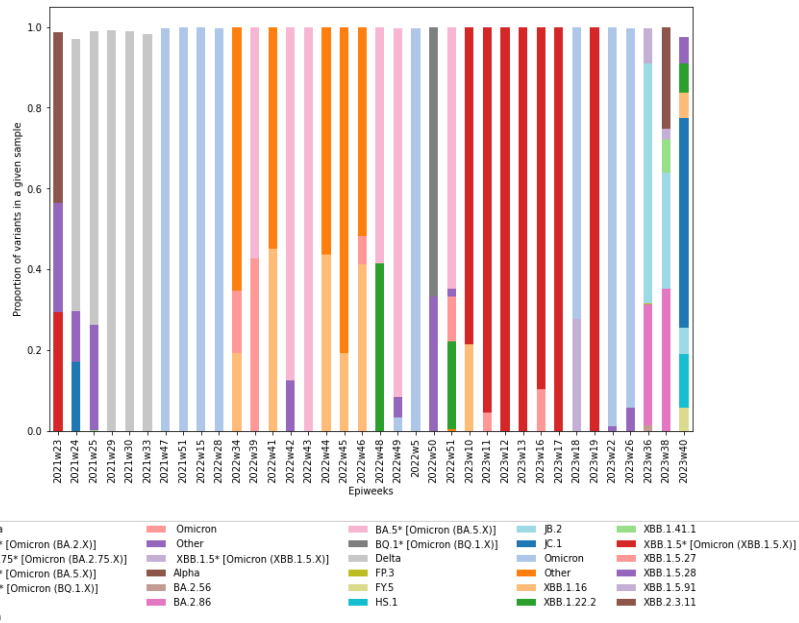
SARS-CoV-2 levels and Genomic Results in Epi week 7:

- The SARS-CoV-2 levels in Hartebeesfontein WWTW decreased in Epi weeks 4 and remain low in Epi week 7.
- As of Epi week 43, there was a slight increase in SARS-CoV-2 levels in Vlakplaats WWTW, after a decrease from Epi week 35. Levels remain low in Epi week 6. No new results are available for Epi week 7.

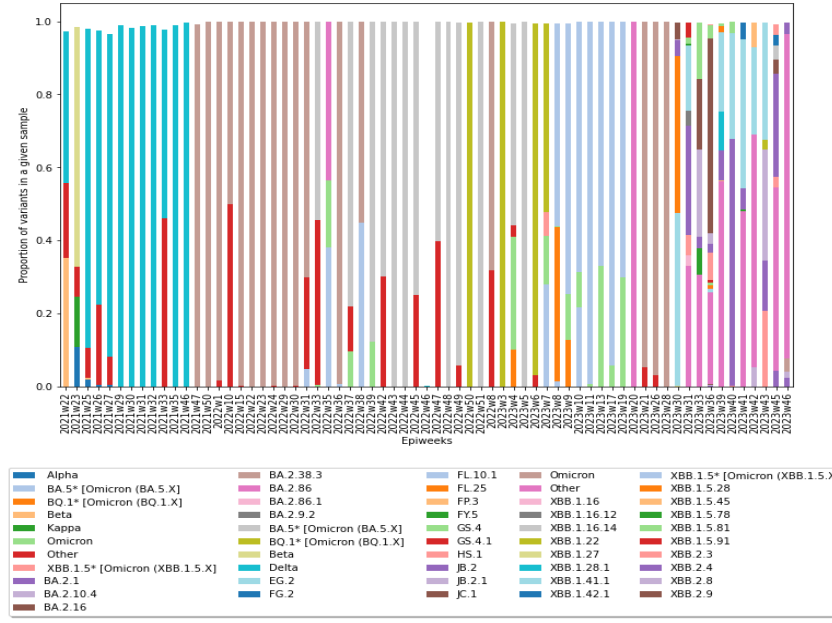
**\* Sequencing data ending in Epi week 40 in Vlakplaats and 46 in Hartebeesfontein.**

- Omicron lineages JC.1, JB.2, XBB.1.41.1, XBB.1.22.2, and XBB.1.5.28 were circulating in Vlakplaats during Epi week 40.
- Lineages BA.2.38.3, XBB.2.4, BA.2.1, BA.2.10.4 and XBB.1.42.1 were circulating during Epi week 46 at the Hartebeesfontein water treatment plant, with BA.2.86 dominating.

ERWAT Vlakplaats Wastewater Treatment Works



Hartebeesfontein Wastewater Treatment Works



SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Ekurhuleni treatment plants.

# KwaZulu-Natal - eThekweni

## SARS-CoV-2 levels and Genomic Results in Epi week 7:

- SARS-CoV-2 levels in Central WWTW in Epi week 6 decrease from Epi week 5, as levels remain low. No new results are available for Epi week 7.
- SARS-CoV-2 levels increased in Epi week 5 in Northern WWTW. There was a slight decrease in levels in Epi week 7, as levels remain low.

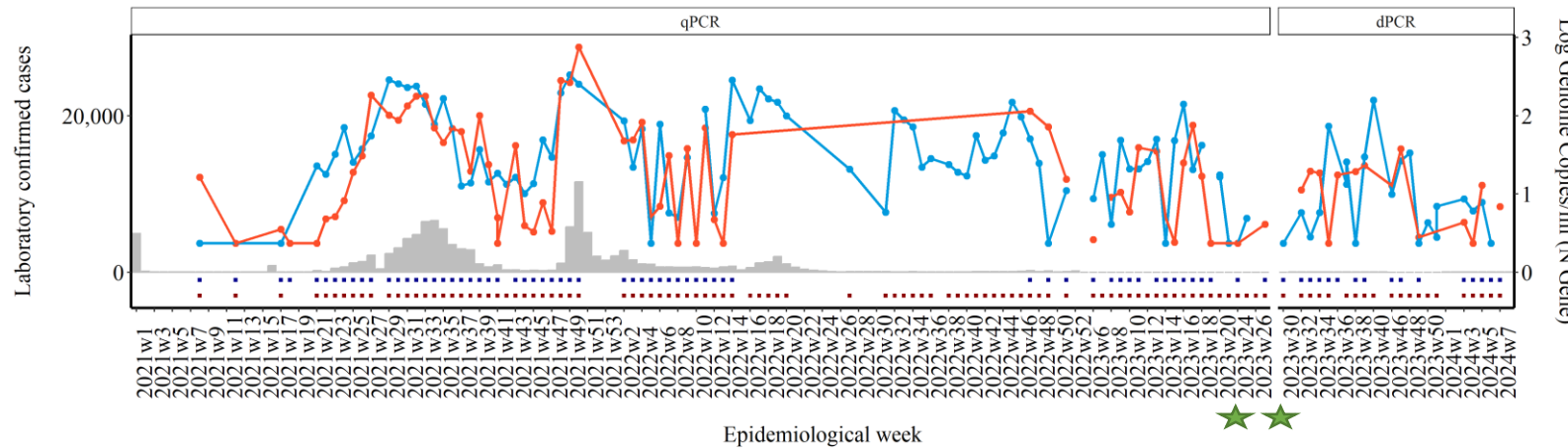
*\* Sequencing data ending in Epi week 46 in Central eThekweni and 46 in eThekweni North.*

- Lineage BA.2.86 was dominating. Other lineages in circulation included: XBB.1.41.1, XBB, and JB.2 in Epi week 46, eThekweni Central.

- In eThekweni North, BA.2.86 was also dominating. In circulation there were also lineages JC.1, GV.1, BA.2.86.1, JB.2, BA.2.10.2, BA.2.23, BA.2.10, and FY.5 during Epi week 46.

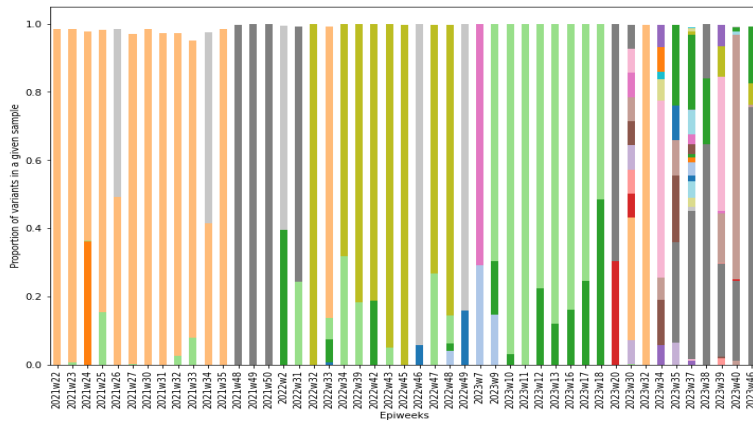
## SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both eThekweni wastewater treatment plants.



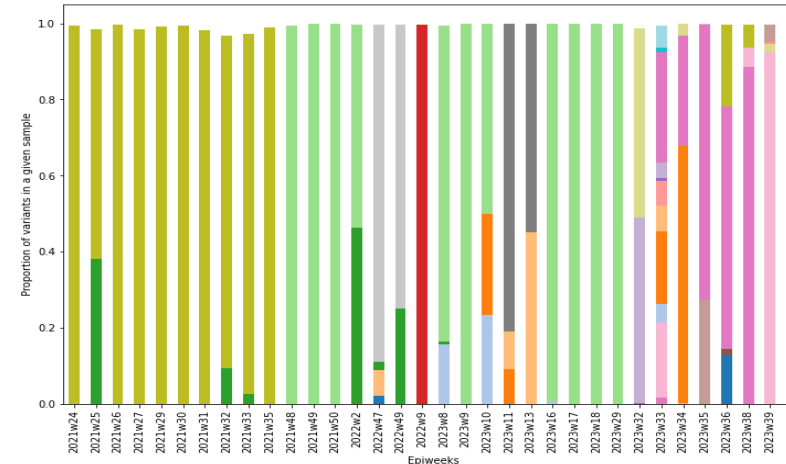
★ Chloroform start and end date    ◆ Central Sample Collection    ■ Central Wastewater Treatment Works (KZN)    ◆ Northern Sample Collection    ■ Northern Wastewater Treatment Works (KZN)

## Central Wastewater Treatment Works



BA.5* [Omicron (BA.5.X)]	BA.2.75* [Omicron (BA.2.75.X)]	FH.1	XBB	XBB.1.5.28
BO.1* [Omicron (BO.1.X)]	BA.2.76	FL.13.2	XBB.1.16	XBB.1.5.32
Beta	BA.2.86	FL.25	XBB.1.16.12	XBB.1.5.42
Delta	BA.5	FP.3	XBB.1.16.17	XBB.1.5.6
Omicron	BA.5* [Omicron (BA.5.X)]	FY.5	XBB.1.16.2	XBB.1.5.60
Other	BM.1.1.1	GR.1	XBB.1.16.20	XBB.1.5.83
XBB.1.5* [Omicron (XBB.1.5.X)]	BM.2.1	HS.1	XBB.1.22.2	XBB.1.5.84
BA.2.1	BM.4	JB.2	XBB.1.41	XBB.2.3.11
BA.2.12	CH.1.1.1	JC.1	XBB.1.41.1	XBK
BA.2.37	CH.1.1.11	JC.1	XBB.1.5* [Omicron (XBB.1.5.X)]	XBL.3
BA.2.38.3	CH.1.1.11	JC.1	XBB.1.5.15	XBU
BA.2.73	Delta	Other	XBB.1.5.17	

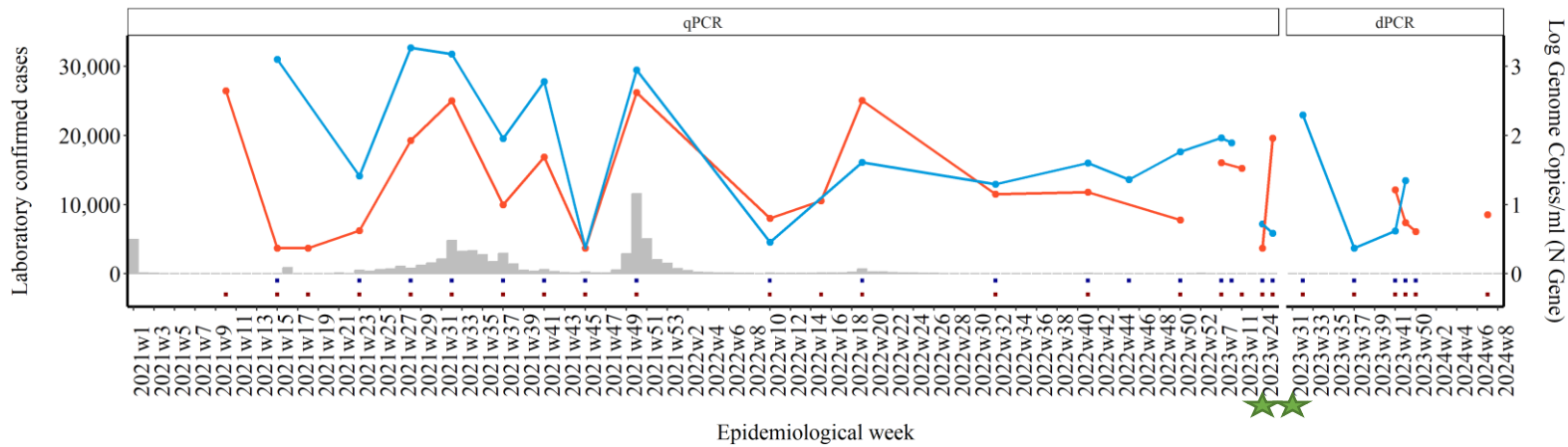
## Northern Wastewater Treatment Works



BA.2* [Omicron (BA.2.X)]	BA.2.15	Delta	JC.1	XBB.1.41.1
BA.5* [Omicron (BA.5.X)]	BA.2.16	FL.25	Omicron	XBB.1.42.1
BO.1* [Omicron (BO.1.X)]	BA.2.23	FY.5	Other	XBB.1.5* [Omicron (XBB.1.5.X)]
Omicron	BA.2.33	GV.1	XBB	XBB.1.5.101
Other	BA.2.74	HK.2	XBB.1.16	XBB.1.5.28
XBB.1.5* [Omicron (XBB.1.5.X)]	BA.2.86	HS.1	XBB.1.16.17	XBB.1.5.81
BA.2.10	BA.2.86.1	JB.2	XBB.1.22.2	XBB.1.5.91
BA.2.10.2	BA.5* [Omicron (BA.5.X)]	JB.2.1	XBB.1.41	XBB.9



# Eastern Cape – Nelson Mandela



★ Chloroform start and end date ■ Brickfield Sample Collection ◆ Brickfield Pre-treatment Works ■ Kwanobuhle Sample Collection ◆ Kwanobuhle Wastewater Treatment Works

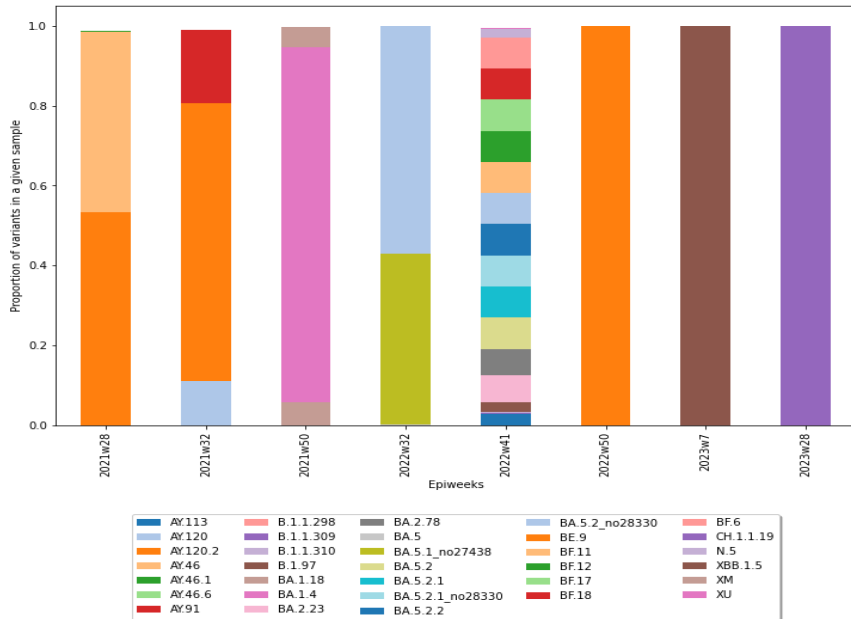
## SARS-CoV-2 levels and Genomic Results in Epi week 7:

- As of Epi week 46, SARS-CoV-2 levels are moderate after an increase was observed after Epi week 36 in the Kwanobuhle WWTW. No new results for Epi week 7 are available.
- SARS-CoV-2 levels decreased from moderate to low from Epi week 43 to Epi week 46 in Brickfield Pre-treatment works. There was a slight increase in level in Epi week 7, but levels remain low.

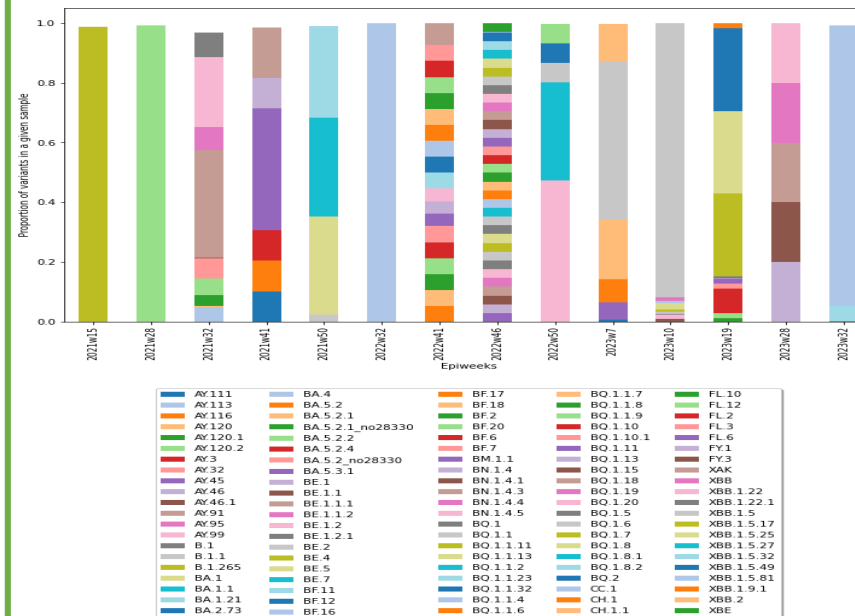
**\* Sequencing data ending in Epi week 28 in Brickfield and 32 in Kwanobuhle. No new sequencing data available.**

- SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-39 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.81, XBB.1.5.32 and XBB.1.5.27 were circulating in Kwanobuhle during Epi week 32.

## Brickfield Pre-treatment works



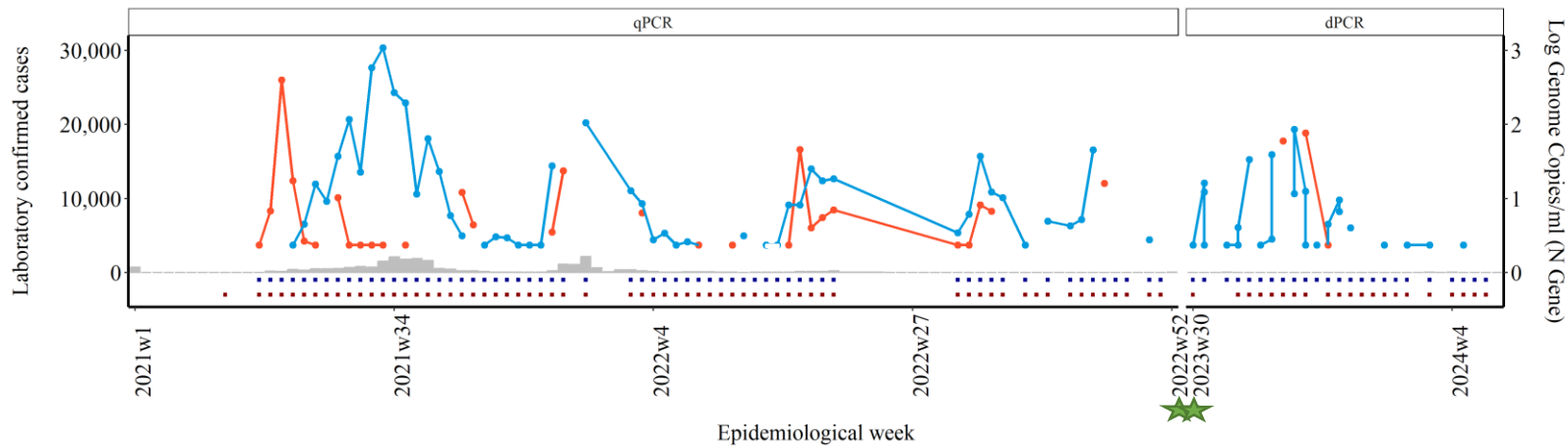
## Kwanobuhle Wastewater Treatment Works



## SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-39 were too low for meaningful interpretation.
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in the Kwanobuhle wastewater treatment plants.

# Eastern Cape – Buffalo City



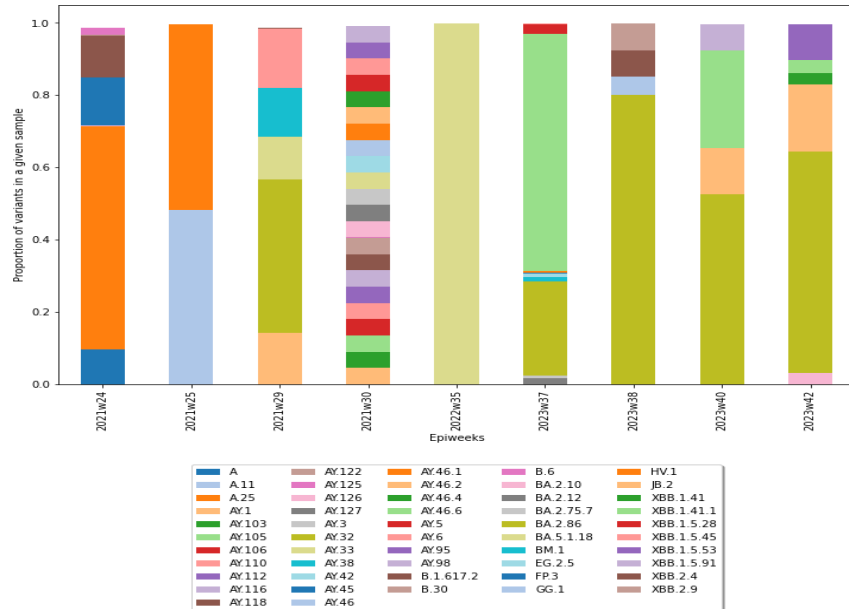
★ Chloroform start and end date ■ East Bank Sample Collection ◆ East Bank Wastewater Treatment Works ■ Mdantsane Sample Collection ◆ Mdantsane Wastewater Treatment Works

## SARS-CoV-2 levels and Genomic Results in Epi week 7:

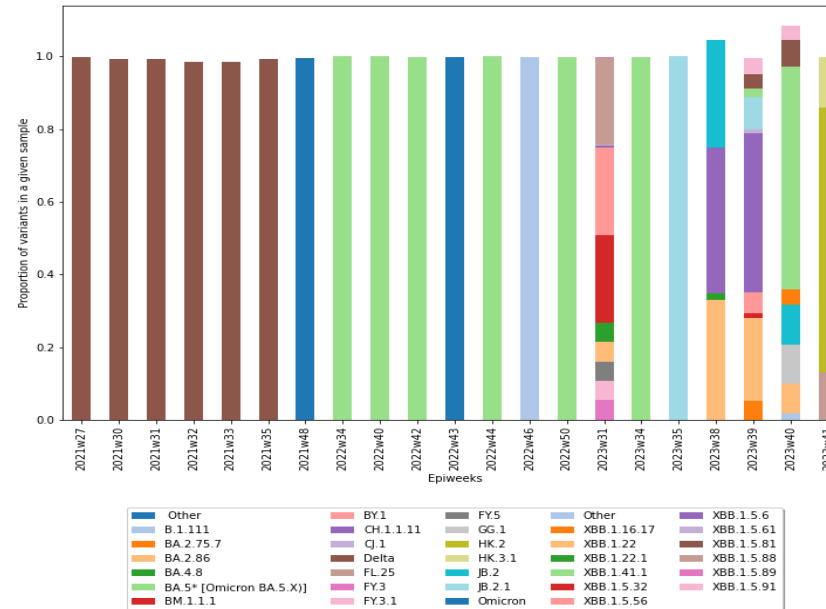
- In Epi week 46, SARS-CoV-2 levels in Mdantsane WWTW decreased and levels are low. Levels in Epi week 5 remain low. No new results for Epi week 7 are available.
- SARS-CoV-2 levels in East Bank WWTW in Epi week 44 remain low after decrease was observed from Epi week 38. No new results for Epi week 7 are available.

**\* Sequencing data ending in Epi week 42 in Eastbank and 41 in Mdantsane.**

## East Bank Wastewater Treatment Works



## Mdantsane Wastewater Treatment Works

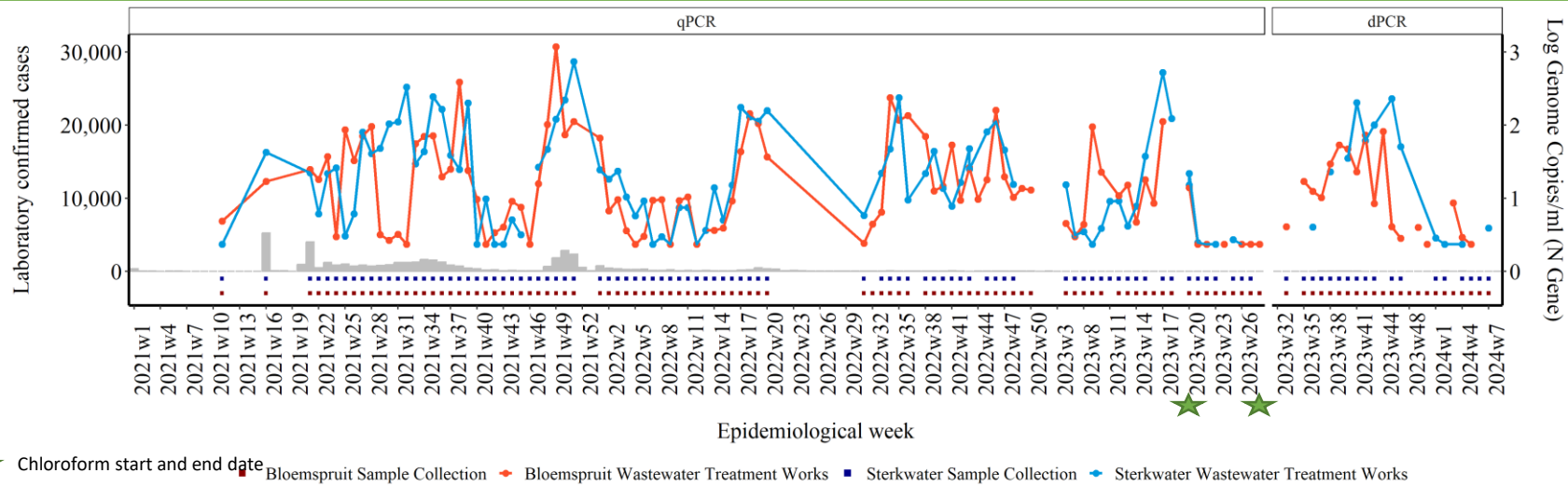


- Omicron lineages BA.2.86, JB.2, XBB.1.5.53 XBB.1.41.1, BA.2.10 and XBB.1.5.91 were circulating in Eastbank during Epi week 42.
- Lineage HK.2 was dominating. HK.3.1 and FL.25, were also circulating in Mdantsane during Epi week 41.

## SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Eastbank and Mdantsane.

# Free State – Mangaung



SARS-CoV-2 levels and Genomic Results in Epi week 7:

- In Bloemspruit WWTW, a 2-fold increase in SARS-CoV-2 levels were seen in Epi week 44. In Epi week 4 and 5 levels decreased and remain low. No new results for Epi week 7 are available.
- A decrease in SARS-CoV-2 levels was seen in Sterkwater WWTW in Epi week 52 and levels remain low in Epi week 4. There is a slight increase in levels in Epi week 7 but levels remain low.

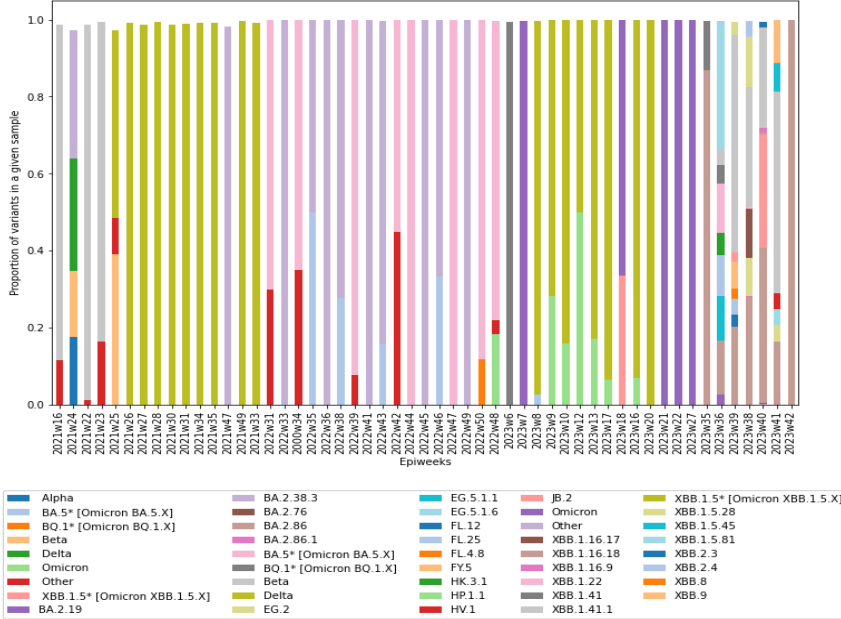
\* Sequencing data ending in Epi week 42 in Bloemspruit and in Epi week 40 in Sterkwater.

- BA.2.86 was the dominant lineage circulating in Bloemspruit during Epi week 42.
- Lineages XBB.1.5.81, JB.2, XBB.1.41.1, and HS.1, were circulating in Sterkwater during epi week 32.

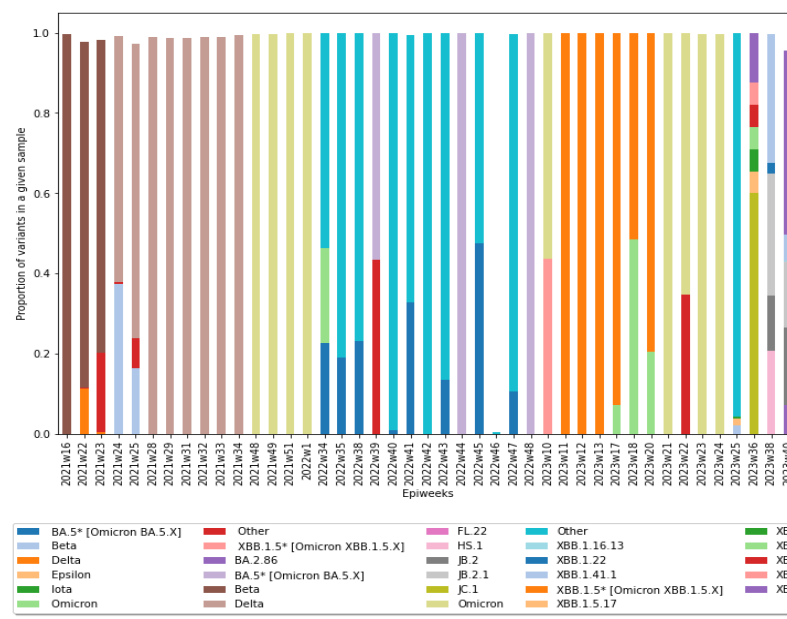
## SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Bloemspruit and Sterkwater.

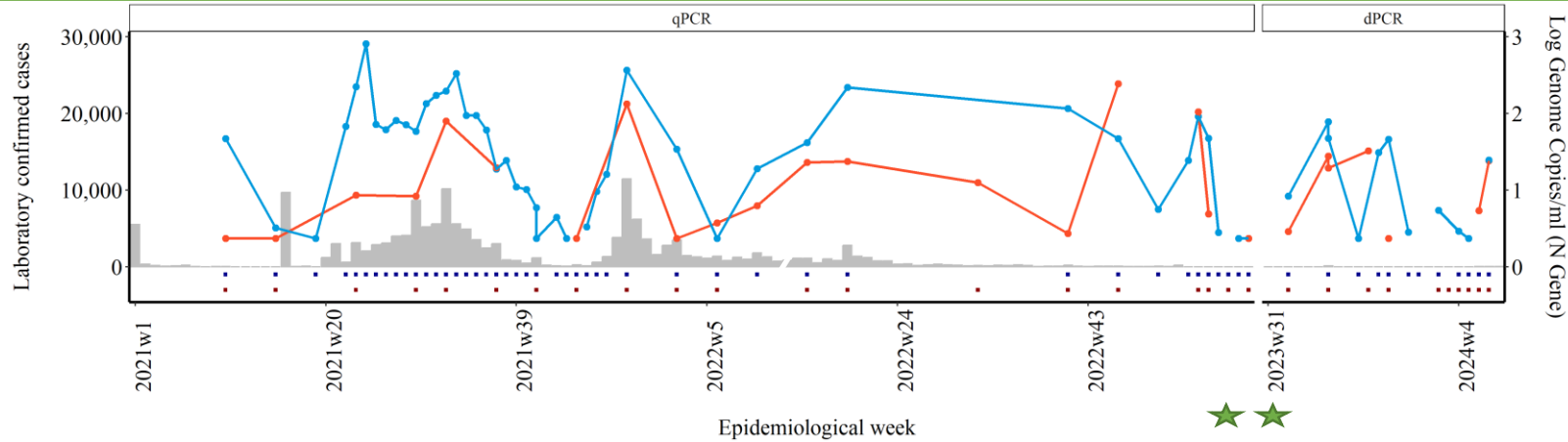
### Bloemspruit Wastewater Treatment Works



### Sterkwater Wastewater Treatment Works



# Western Cape – City of Cape Town



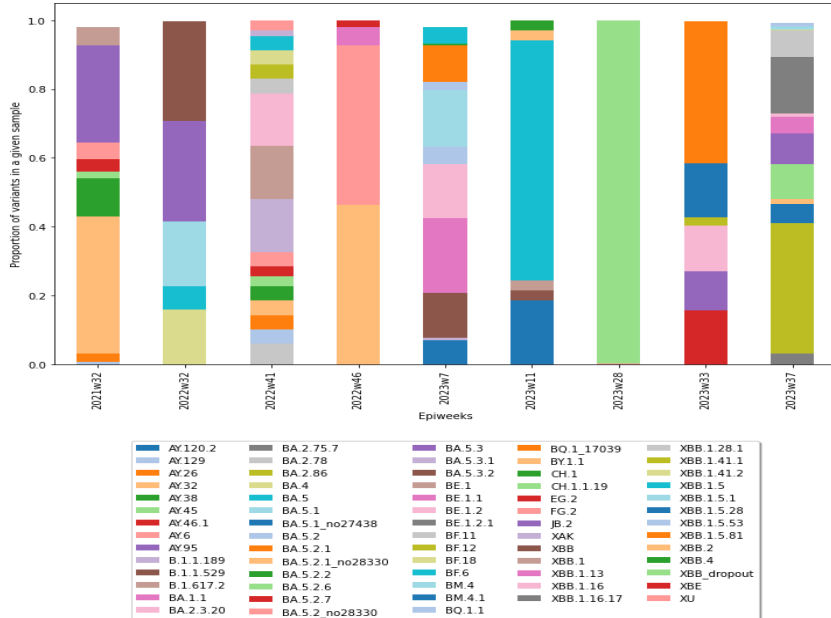
SARS-CoV-2 levels and Genomic Results in Epi week 7:

- After a sharp increase in SARS-CoV-2 levels was seen in Epi week 37, a subsequent decrease in SARS-CoV-2 levels in Borcheds Quarry WWTW was seen. Levels remain low in Epi week 49. Levels are low in Epi week 6 and increase to moderate in Epi week 7.
- In Epi week 4, SARS-CoV-2 levels were low at Zandvleit WWTW. Levels decrease slightly in Epi week 5. In Epi week 7 levels increase to moderate.

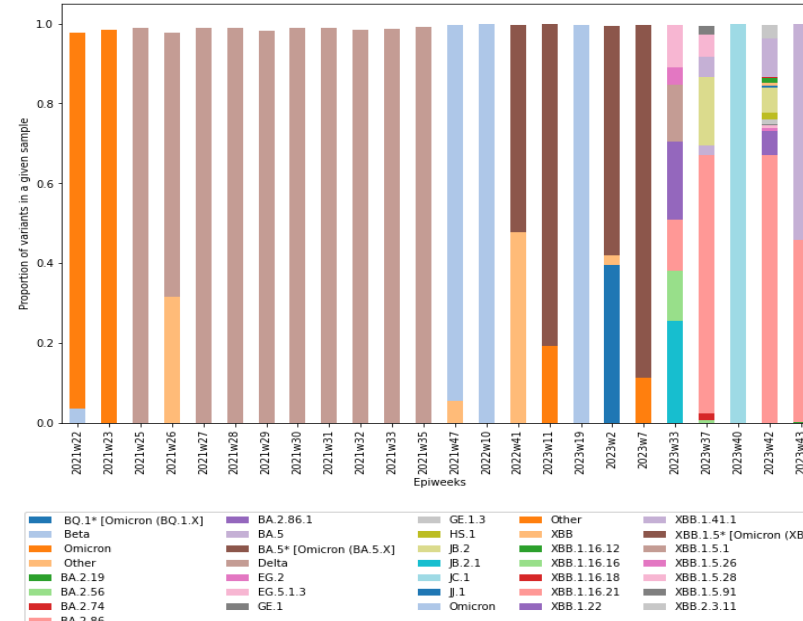
**\* Sequencing data ending in Epi week 37 in Borcheds Quarry and Epiweek 43 in Zandvleit.**

★ Chloroform start and end date   ■ Borcheds Quarry Sample Collection   ◆ Borcheds Quarry Wastewater Treatment Works   ■ Zandvleit Sample Collection   ◆ Zandvleit Wastewater Treatment Works

**Borcheds Quarry Wastewater Treatment Works**



**Zandvleit Wastewater Treatment Works**



- During epiweek 37, Omicron lineages BA.2.86, CH.1.1.19, XBB.1.28.1, XBB.1.16, and JB.2 were circulating in Borcheds Quarry.
- During Epi week 43, lineages BA.2.86 and XBB.1.41.1 were dominant in Zandvleit.

SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Borcheds Quarry and Zandvleit.

# COLLABORATORS



# FUNDERS



# TEAM

