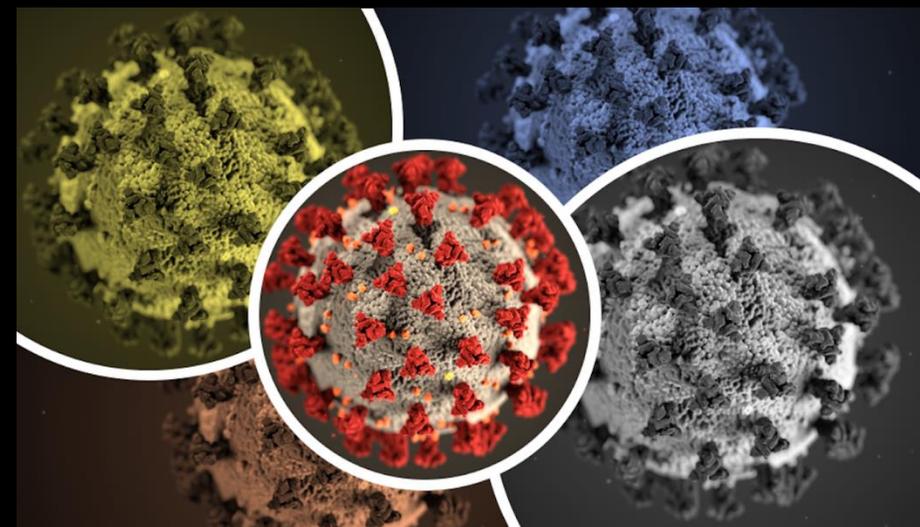


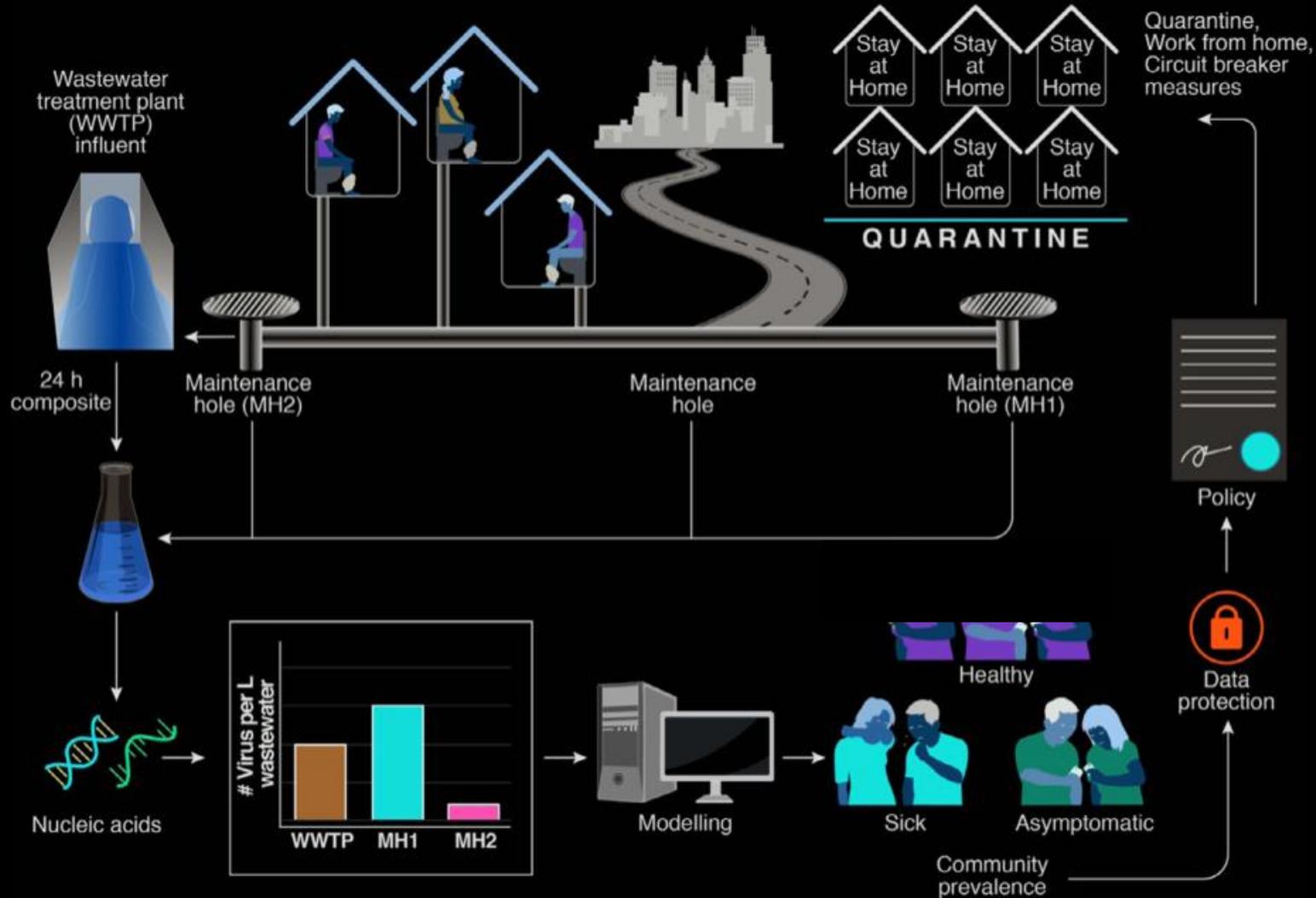
# Auf den Spuren von SARS-CoV-2 in Abwasser – Entwicklungen bei der Sequenzierung von Abwasserproben

Prof. Susanne Lackner  
Dr. rer.nat Laura Orschler  
Dr.-Ing. Shelesh Agrawal



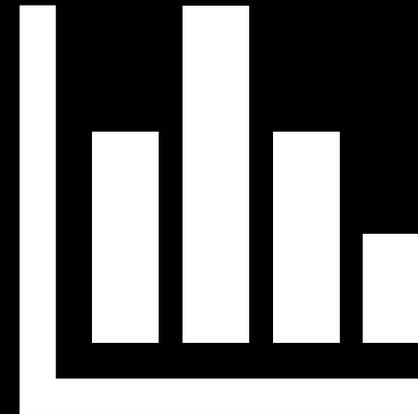
Institut IWAR, Fachgebiet Wasser und Umweltbiotechnologie  
Technische Universität Darmstadt

# SARS-CoV-2 im Abwasser → Monitoringpotentiale?



# Welche Informationen lassen sich mit Abwasseranalysen generieren ?

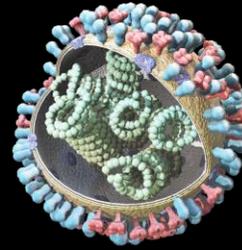
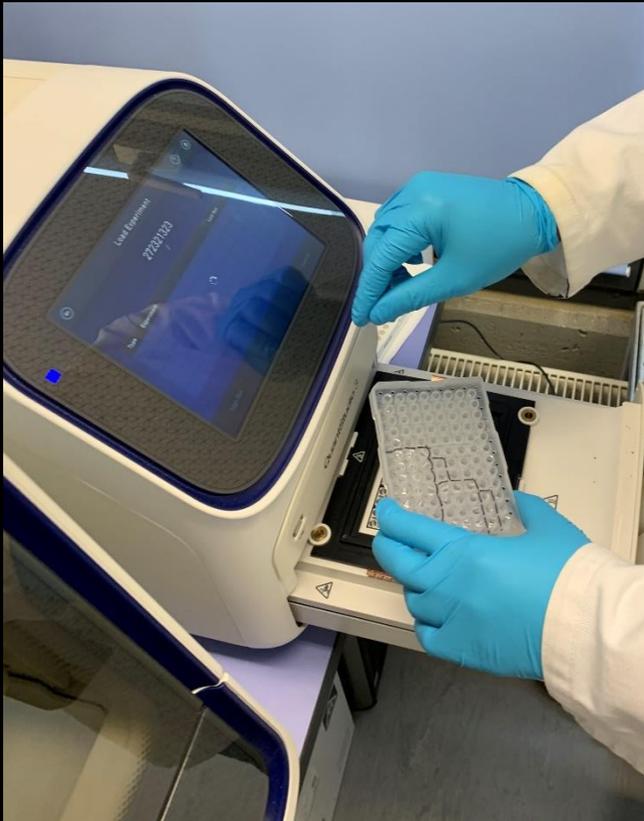
## Trends



## Detektion von Mutationen



Quantitativer Nachweis  
mit qPCR / dPCR



Gene

AA Mutationen

E  
N  
Orf  
M  
...  
...  
...  
...

N501Y  
Del69/70  
P681R  
D950N  
D614G  
...  
...  
...  
...



Nachweis von Mutationen zur  
Charakterisierung der VOC  
mittels Sequenzierung



# Welche Möglichkeiten bietet die Sequenzierung

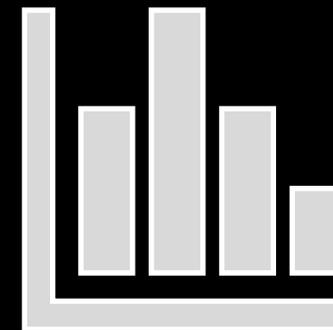


## Mutationsnachweis:

- Nachweis von Mutationsspektren
- Auswertung von Mutationsprofilen
- Nachweis unbekannter Mutationen

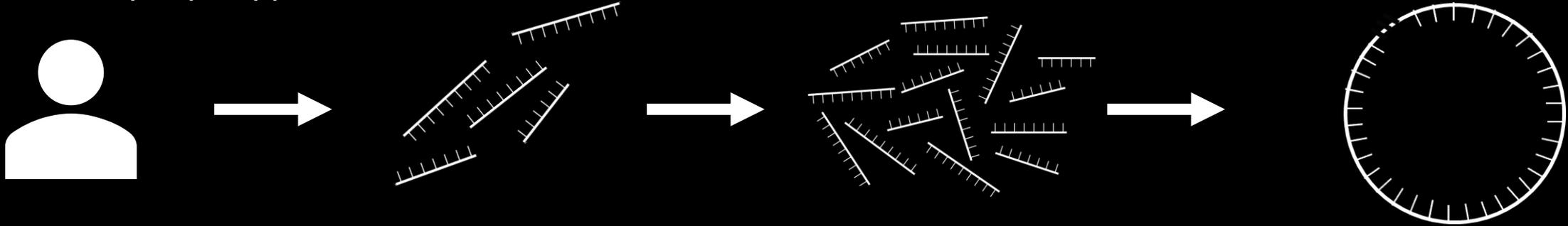
## Variantennachweis:

- Nachweis von VOCs
- Nachweis von Subtypen
- Nachweis sonstiger Varianten



# Genome Sequencing approach

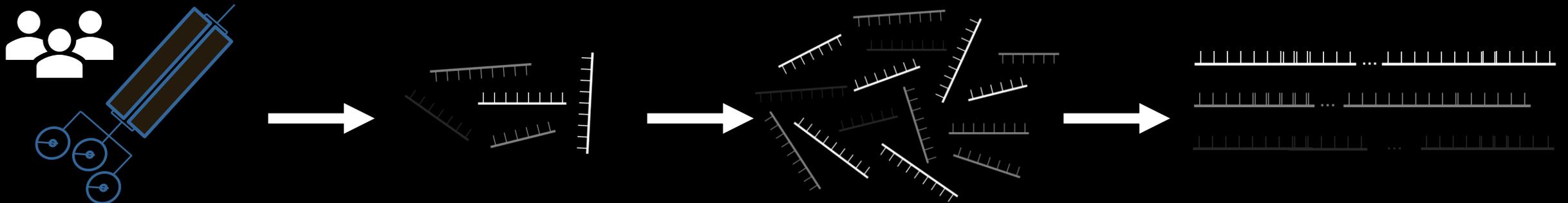
human sample (swap)



RNA extraction and amplification

genome sequencing data

raw wastewater sample

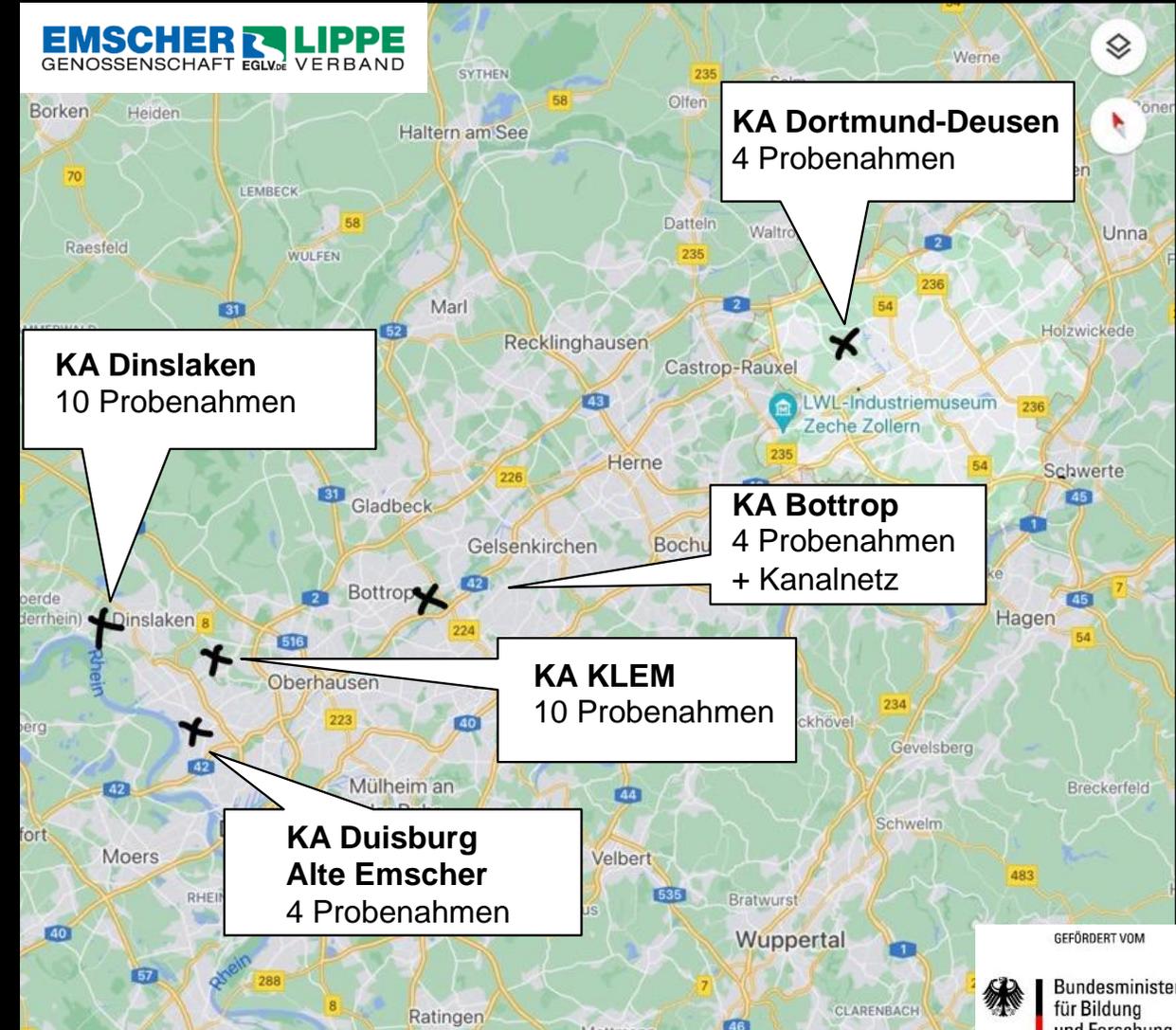


# Beispiel 1: BMBF – Vorhaben SARS-GenA-Seq



Das SARS-CoV-2 Genom im Abwasser –  
Monitoring der Pandemieentwicklung  
mittels Sequenzierung

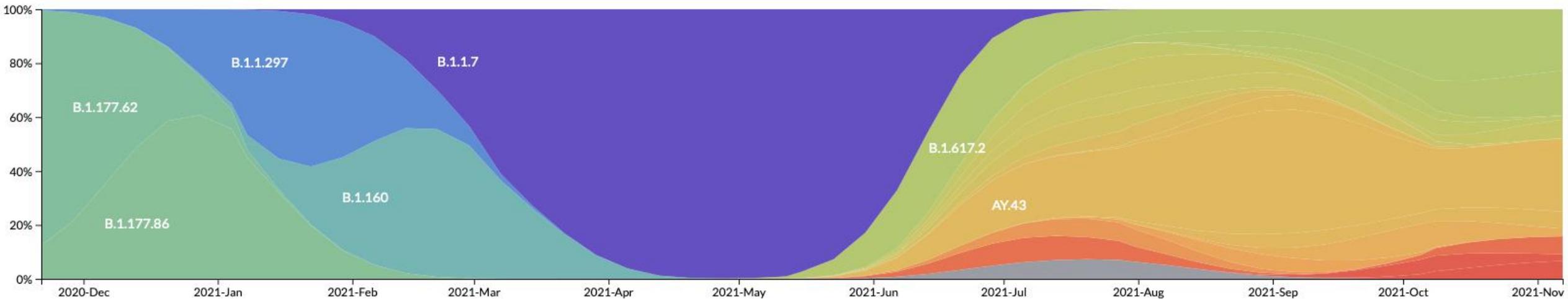
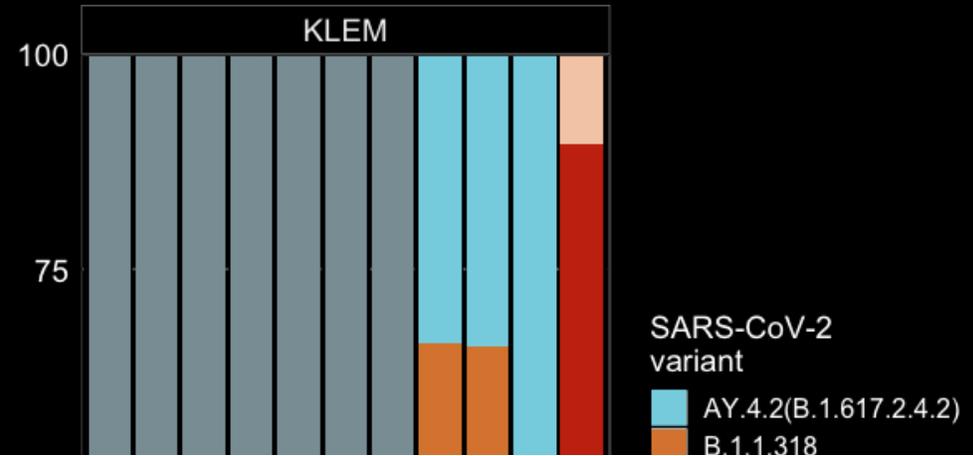
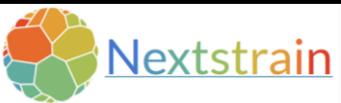
- Probenahmen im Emscher-Einzugsgebiet seit April 2021
- Fragestellungen:
  - Probennahme, -Volumen, Umwelteinflüsse
  - Sequenzierungs-Methoden
  - Bioinformatische Auswertung



# Klärwerk Emschermündung (KLEM)

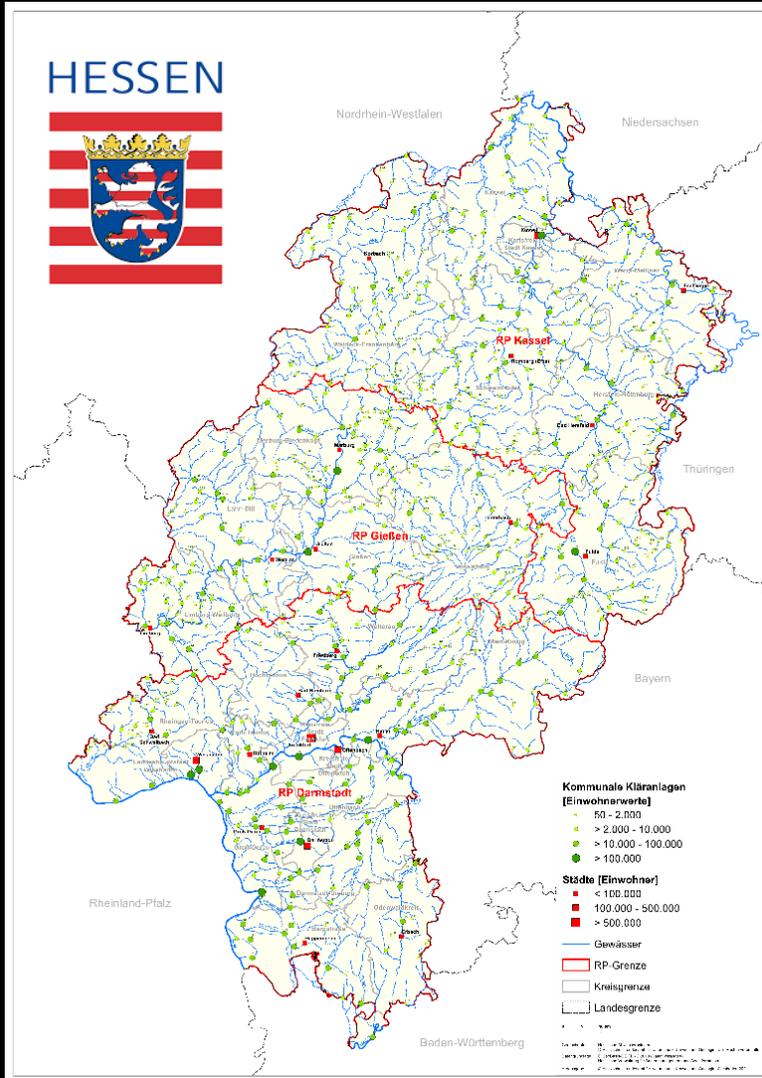


- wenig Diversität im Mai / Juni 2021
- ab Okt. 2021 mehr Varianten nachweisbar
- AY 4.2 machte im Sept/Okt ca. 50 % der Delta-Nachweise aus
- Omikron ab Dezember 2021 nachweisbar



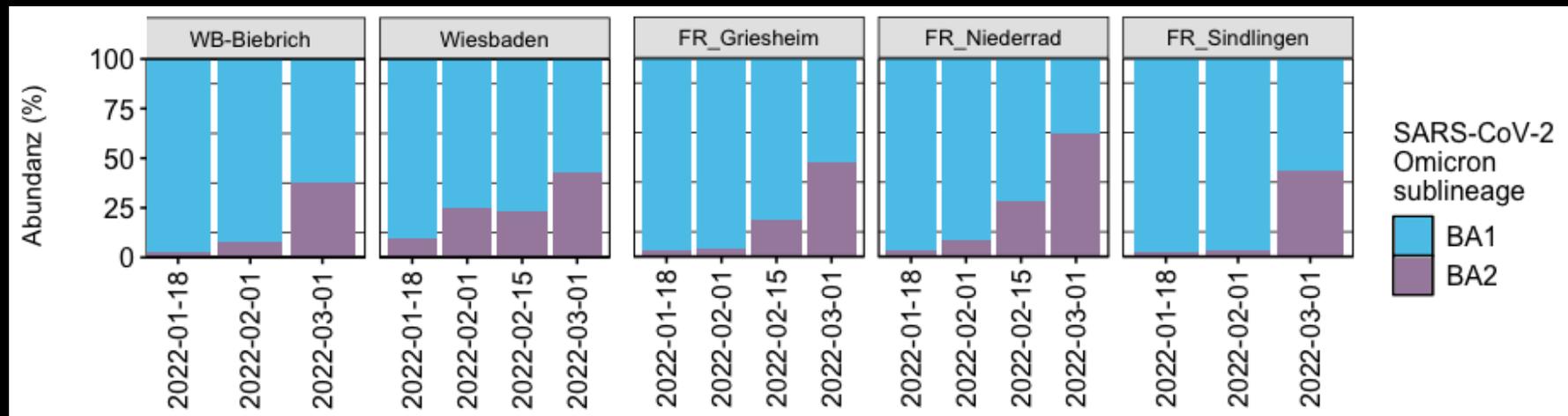
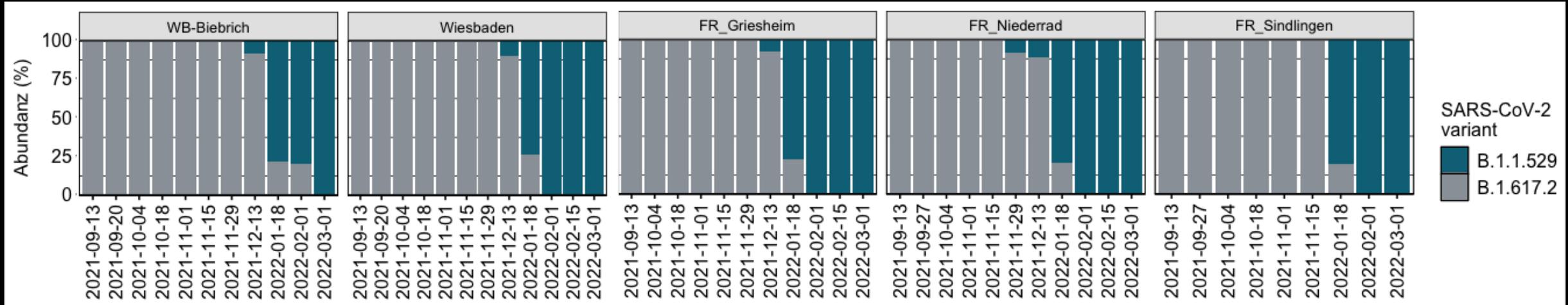
2021-04  
2021-05  
2021-05  
2021-05  
2021-05  
2021-06  
2021-06  
2021-09  
2021-10  
2021-10  
2021-12

# Beispiel 2: Hessenweites Monitoring von Varianten

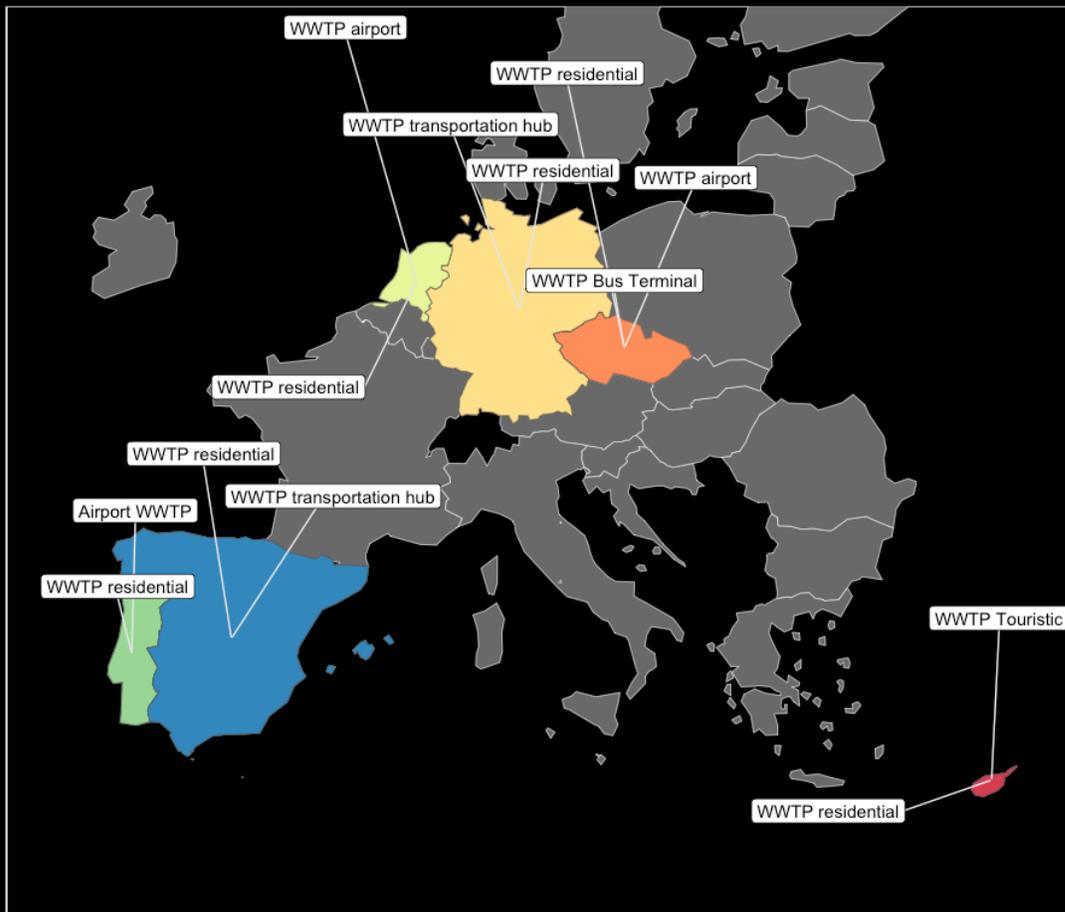


- Ganzheitliches Konzept zur Nachverfolgung von SARS-CoV-2 Varianten in Hessen (HeNaSARS-V)
- 18 Standorte in ganz Hessen
- Sequenzierung von Proben im Abstand von 2 - 4 Wochen
- Laufzeit: Sept. 2021 – April 2022
- Zusammenarbeit mit dem Hessisches Landesprüfungs- und Untersuchungsamt im Gesundheitswesen (HLPUG) etablieren

# Verläufe in Frankfurt und Wiesbaden



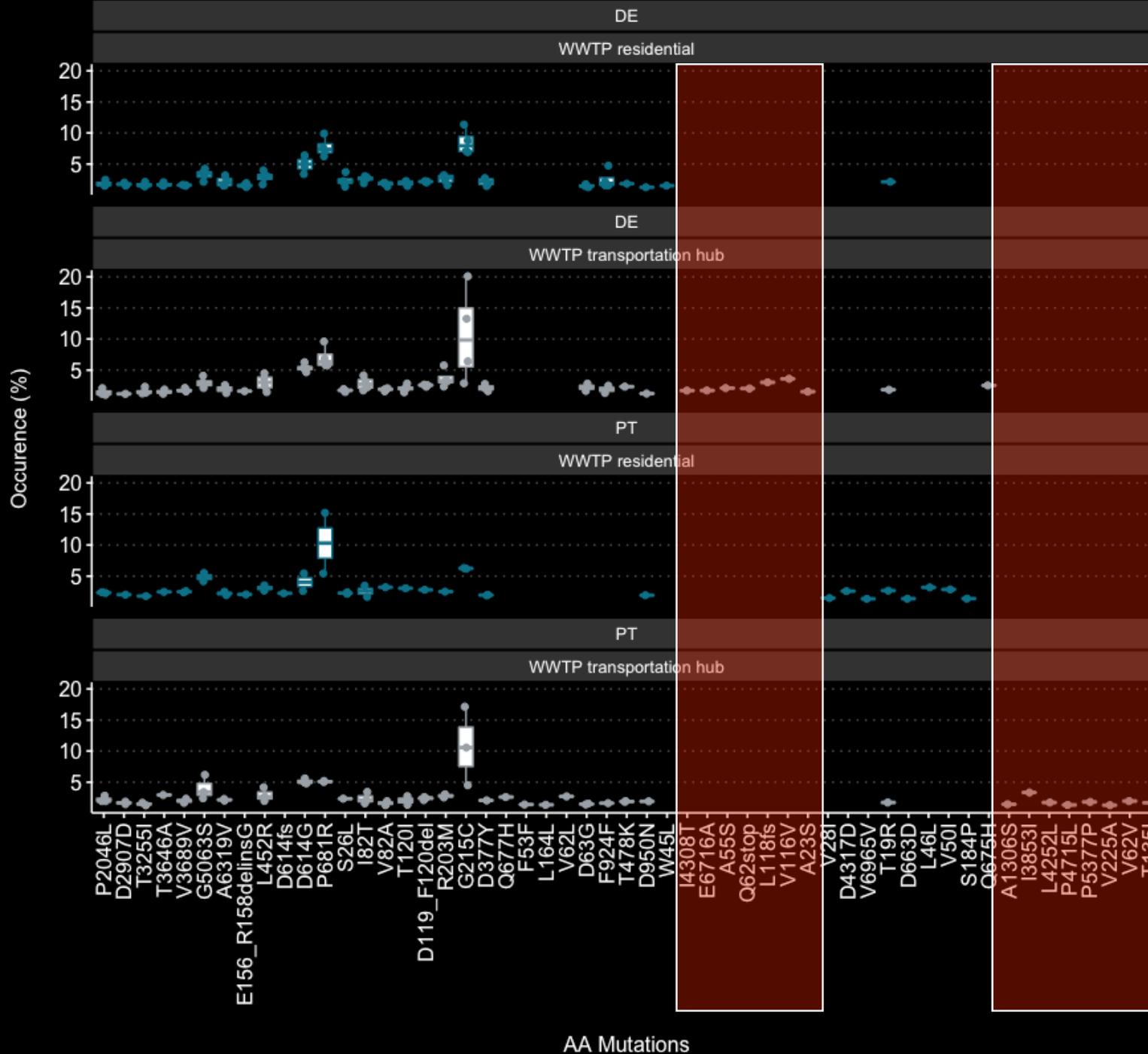
# Beispiel 3: EU SuperSites



- Country
- Cyprus
  - Czechia
  - Germany
  - Netherlands
  - Portugal
  - Spain

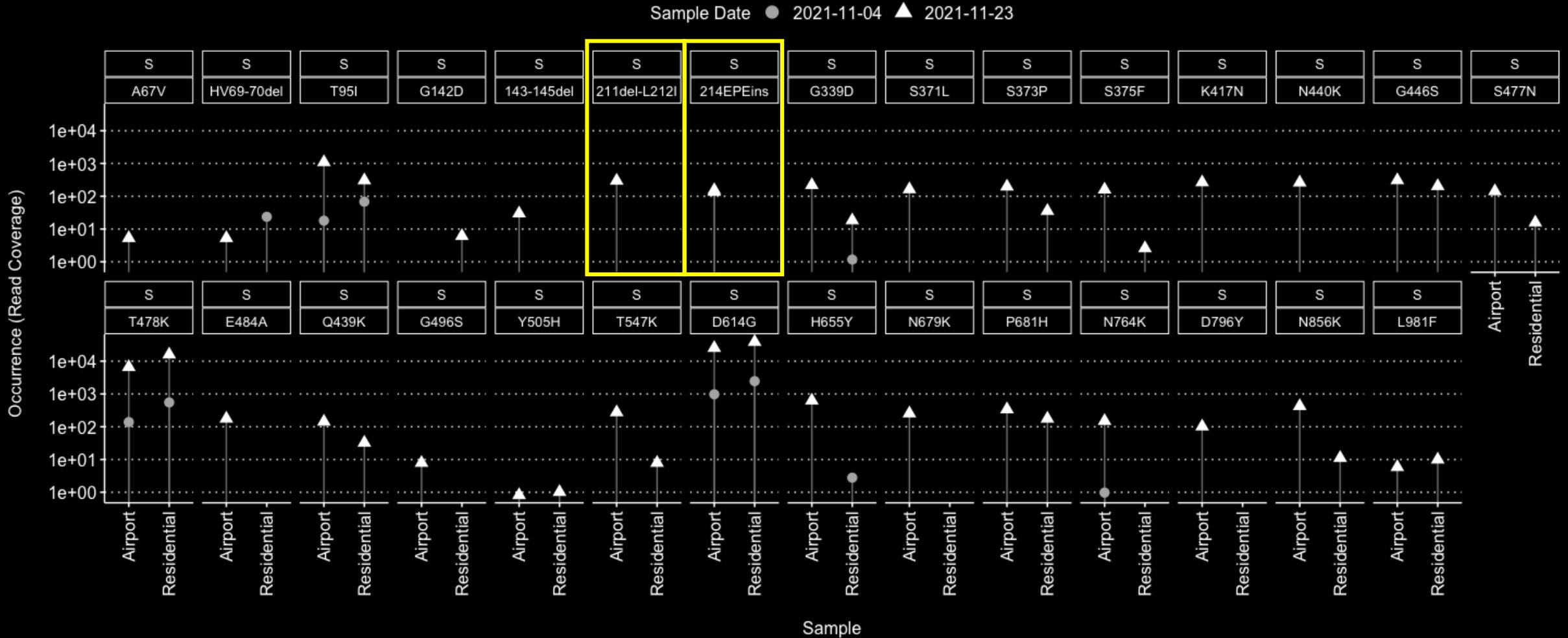


Does Wastewater surveillance of SuperSites reveal a different story?

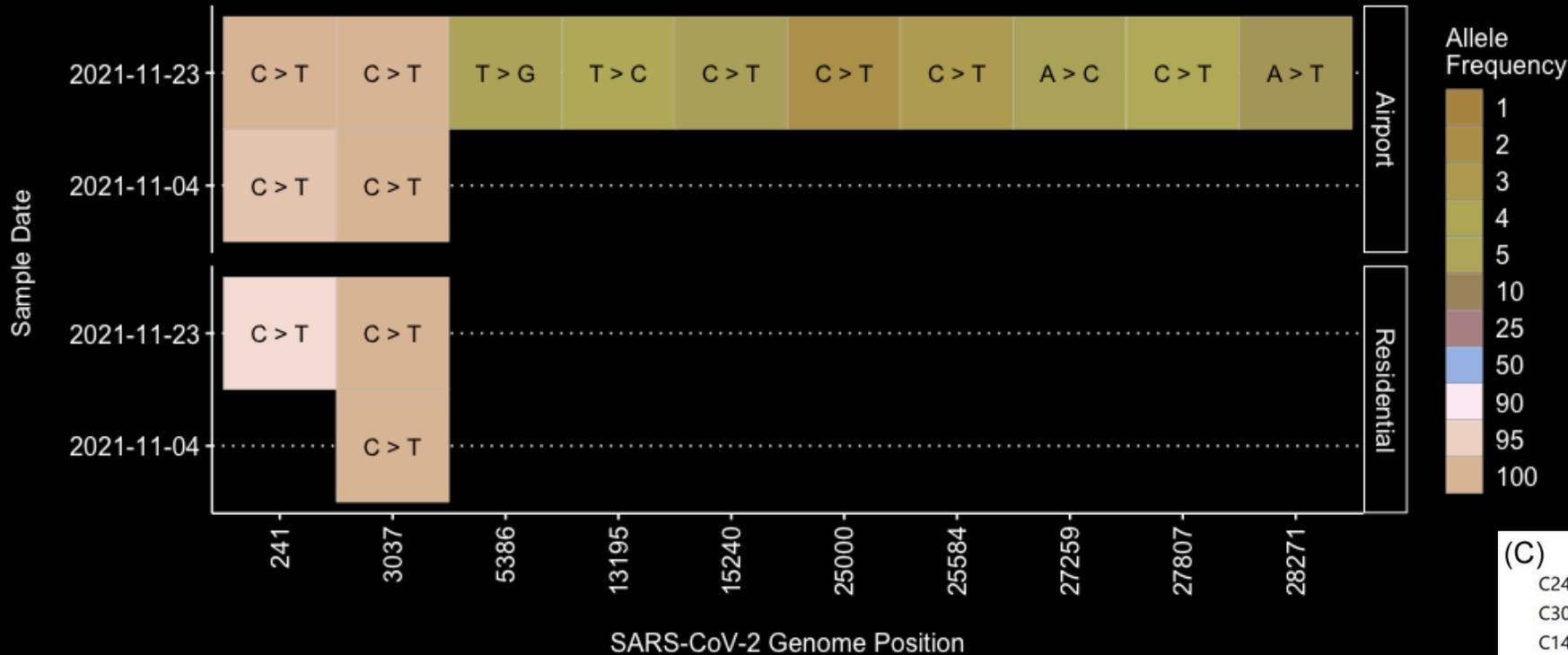


among the top 20  
mutations some  
mutations are specific to  
certain SuperSites

# Nachweis von Omikron in Frankfurt



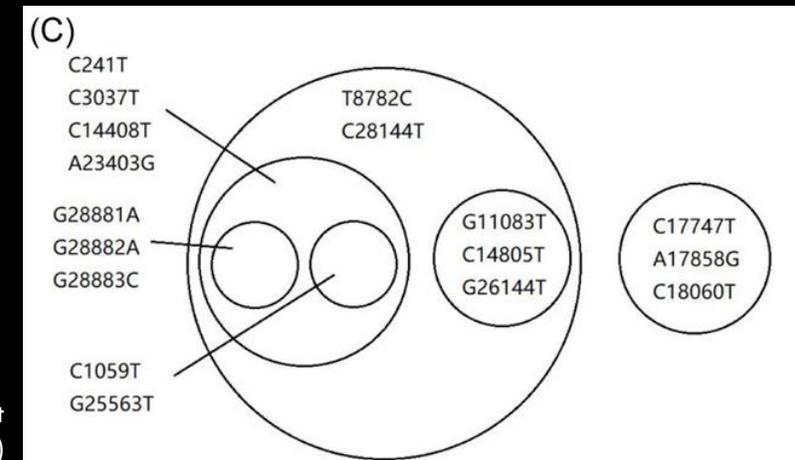
# Nachweis von Omikron aus weiteren Veränderungen in der Genomsequenz



Erkenntnisse über die Virus Evolution auch aus stillen Mutationen

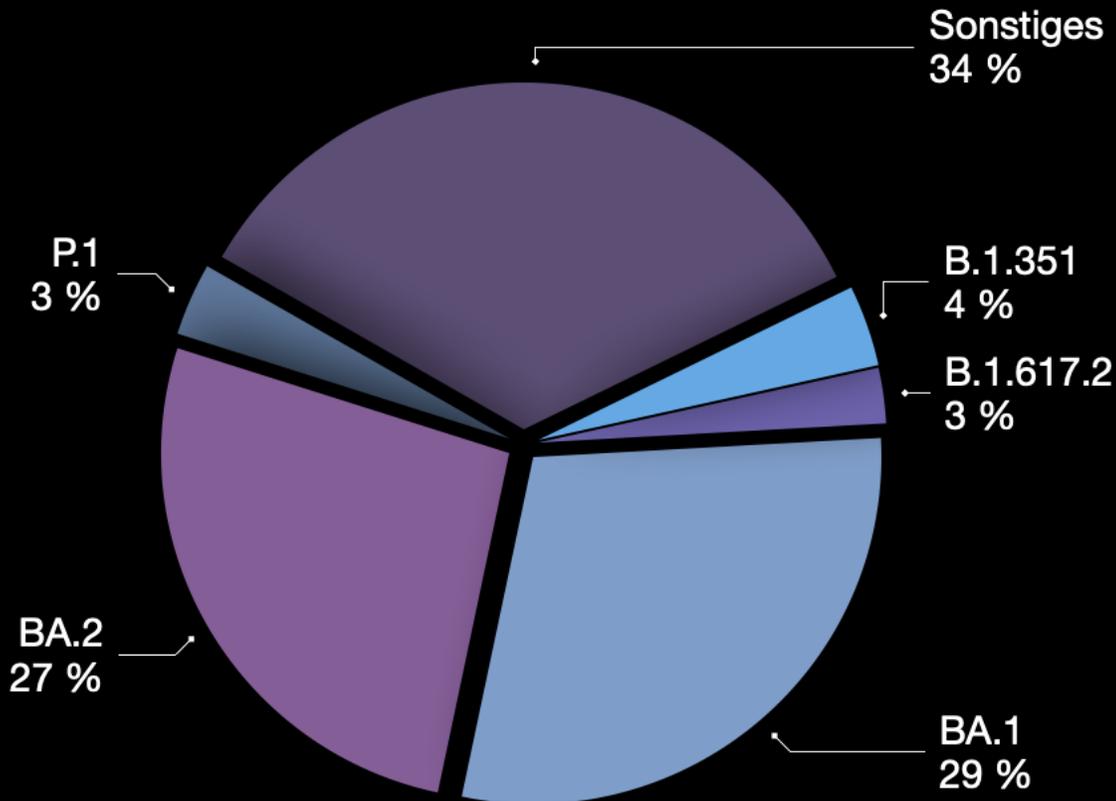
Synonyme Mutationen – kodiert die gleiche Aminosäure

Venn diagram showing the subsequence relationships of the 17 most frequent mutations (Chen et al. 2022)



# Das Potential unbekannter Mutationen

Etwa 34 % der AA-Mutationen in dieser Abwasserprobe waren nicht mit den aktuellen VOCs assoziiert




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ARTICLE  
<https://doi.org/10.1038/s41467-022-28246-3> OPEN

## Tracking cryptic SARS-CoV-2 in NYC wastewater

David S. Smyth<sup>1,6</sup>, Monica Trujillo<sup>2,6</sup>, Devon A. Gre Yue Guan<sup>3</sup>, Caitlyn Guldenpfennig<sup>3</sup>, Irene Hoxie<sup>4</sup>, S Michelle Markman<sup>4</sup>, Clayton Rushford<sup>3</sup>, Kaung Myat Reinier Suarez<sup>3</sup>, Emma Teixeira<sup>3</sup>, Mark Daniels<sup>3</sup>, Marc

### Highly divergent white-tailed deer SARS-CoV-2 with potential deer-to-human transmission

Bradley Pickering<sup>1,9,10</sup>, Oliver Lung<sup>1,11</sup>, Finlay Maguire<sup>2,12,13</sup>, Peter Kruczkiewicz<sup>2</sup>, Jonathan D. Kotwa<sup>3</sup>, Tore Buchanan<sup>4</sup>, Marianne Gagnier<sup>5</sup>, Jennifer L. Guthrie<sup>6,16</sup>, Claire M. Jardine<sup>7</sup>, Alex Marchand-Austin<sup>6</sup>, Ariane Massé<sup>5</sup>, Heather McClinchey<sup>8</sup>, Kuganya Nirmalarajah<sup>3</sup>, Patryk Aftanas<sup>13</sup>, Juliette Blais-Savoie<sup>3</sup>, Hsien-Yao Chee<sup>3</sup>, Emily Chien<sup>3</sup>, Winfield Yim<sup>3</sup>, Melissa Goolia<sup>1</sup>, Matthew Suderman<sup>1</sup>, Mathieu Pinette<sup>1</sup>, Greg Smith<sup>1</sup>, Daniel Sullivan<sup>1,11</sup>, Jossip Rudar<sup>1</sup>, Elizabeth Adey<sup>1</sup>, Michelle Nebroski<sup>1</sup>, Marcelline Côté<sup>17,18,19</sup>, Geneviève Laroche<sup>17,18,19</sup>, Allison J. McGeer<sup>14,20</sup>, Larissa Nituch<sup>4</sup>, Samira Mubareka<sup>3,14</sup>, Jeff Bowmar<sup>4,15</sup>

<sup>1</sup>National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada  
<sup>2</sup>Faculty of Computer Science, Dalhousie University, Nova Scotia, Canada  
<sup>3</sup>Sunnybrook Research Institute, Toronto, Ontario, Canada  
<sup>4</sup>Wildlife Research and Monitoring Section, Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry, Peterborough, Ontario, Canada  
<sup>5</sup>Ministère des Forêts, de la Faune et des Parcs, Québec City, Québec, Canada  
<sup>6</sup>Public Health Ontario, Toronto, Ontario, Canada  
<sup>7</sup>Canadian Wildlife Health Cooperative, Ontario-Nunavut, Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada  
<sup>8</sup>Public Health, Health Protection and Surveillance Policy and Programs Branch, Ontario Ministry of Health  
<sup>9</sup>Department of Veterinary Microbiology and Preventative Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA  
<sup>10</sup>Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg  
<sup>11</sup>Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada  
<sup>12</sup>Department of Community Health & Epidemiology, Faculty of Medicine, Dalhousie University, Nova Scotia, Canada  
<sup>13</sup>Shared Hospital Laboratory, Toronto, Ontario, Canada  
<sup>14</sup>Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada  
<sup>15</sup>Environmental and Life Sciences Graduate Program, Trent University, Ontario, Canada  
<sup>16</sup>Department of Microbiology & Immunology, Western University, London, Ontario, Canada

Tracking SARS-CoV-2 genetic diversity is strongly indicated by... may lead to the emergence of novel variants resistant to naturally induced immunity. To monitor New York City (NYC) for the presence of deep sequence most of the receptor binding domain coding sequences SARS-CoV-2 isolated from the New York City wastewater. We observed increasing frequencies of novel cryptic SARS-CoV-2 lineages in the EpiCoV database. These lineages contain mutations that had been absent in samples, including Q493K, Q498Y, E484A, and T572N and shared with the Omicron variant of concern. Some of these mutations expand the receptor binding domain by allowing infection of cells expressing the ACE2 receptor. Finally, pseudoviruses containing the spike amino acid sequence were resistant to different classes of receptor binding domain antibodies. We offer several hypotheses for the anomalous findings including the possibility that these lineages are derived from unknown infections or that they indicate the presence of a non-human animal source.

HESSSEN



GEFÖRDERT VOM



Bundesministerium  
für Bildung  
und Forschung



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COMMISSION



Vielen Dank!

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