



Bridging the gaps in bioinformatics

The role of genome sequencing in pathogen surveillance

Statens Serum Institut (SSI)

SSI

- Is under the auspices of the Danish Ministry of Health. Main duty is to ensure preparedness against infectious diseases and biological threats, and control congenital disorders and provide counselling to the Danish healthcare system and authorities in the event of epidemics
- Specialized Diagnostics
- House Danish National Biobank which stores more than 22 million biological samples
- Is responsible for the national screening of newborns
- Research
 - All of SSI's activities are based on research
- Vaccine supply
 - SSI is responsible for the purchase and supply of vaccines to the Danish national vaccination programmes, including the Childhood Vaccination Programme, pandemic preparedness and other necessary preparedness supplies

Statens Serum Institut

The Department of Bacteria, Parasites and Fungi

- Responsible for national public health activities and performs surveillance and specialized diagnostic analysis in relation to bacteria, parasites and fungi

Surveillance of antimicrobial resistance in bacteria and fungi and the consumption of antimicrobials in the primary health care sector and hospitals in Denmark (DANMAP)



DANMAP 2017

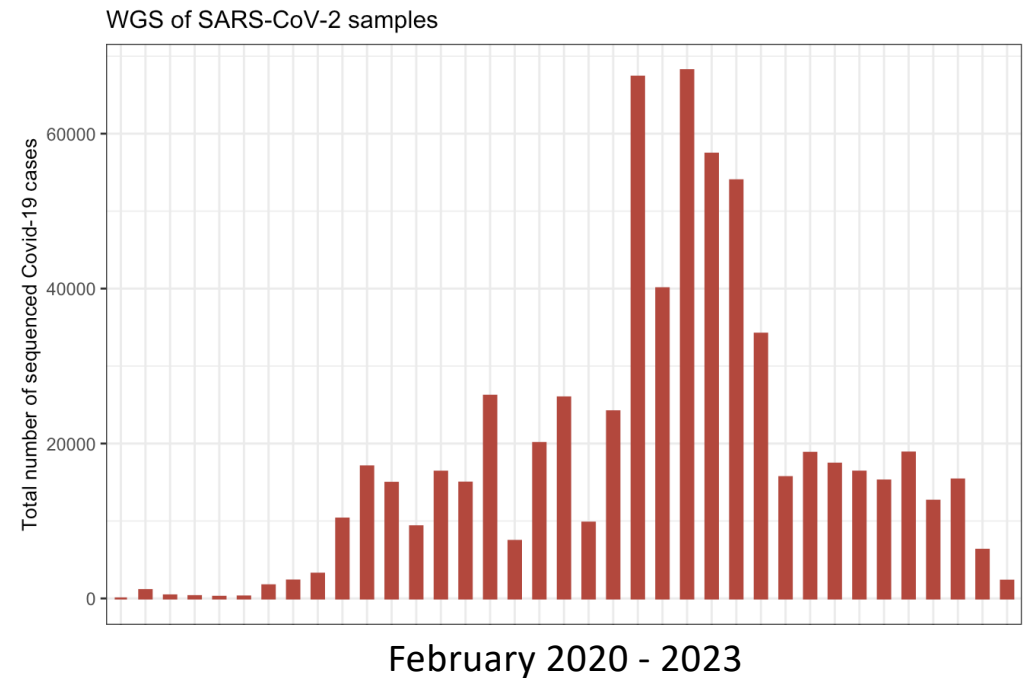
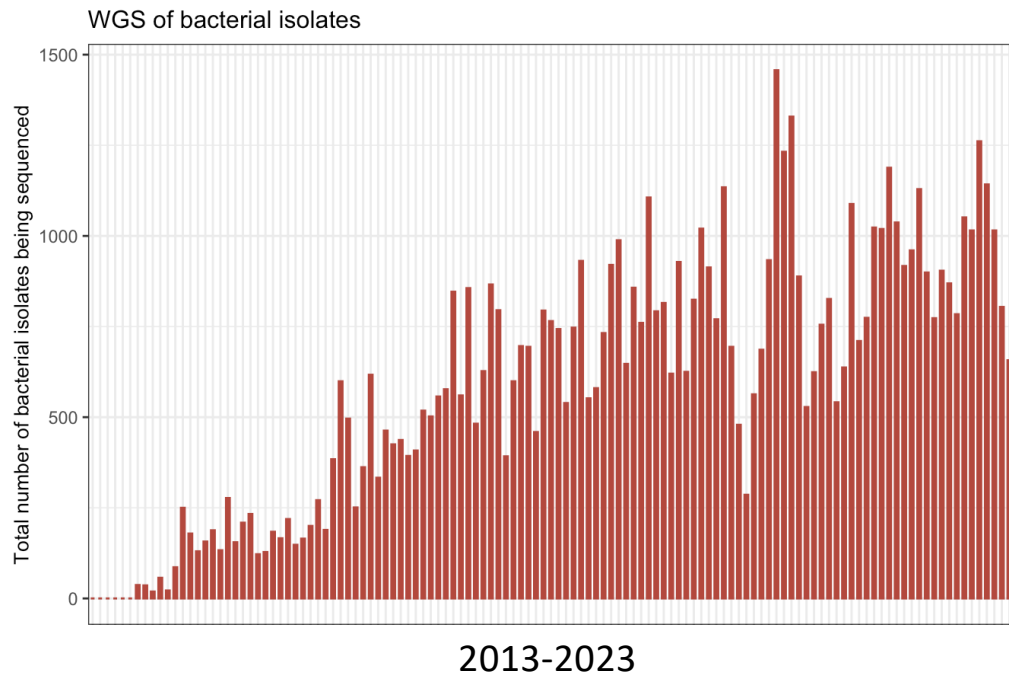
DANMAP 2017 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark



Statens Serum Institut
National Veterinary Institute, Technical University of Denmark
National Food Institute, Technical University of Denmark

Section for Sequencing and Bioinformatics at SSI

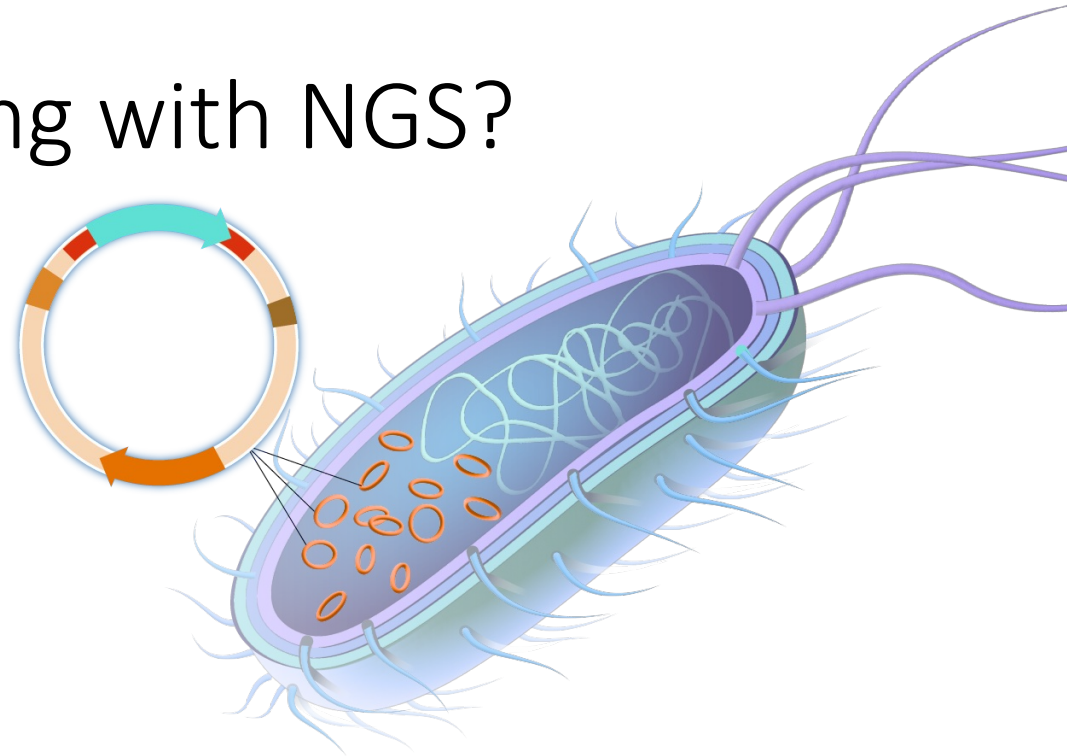
WGS at Statens Serum Institut



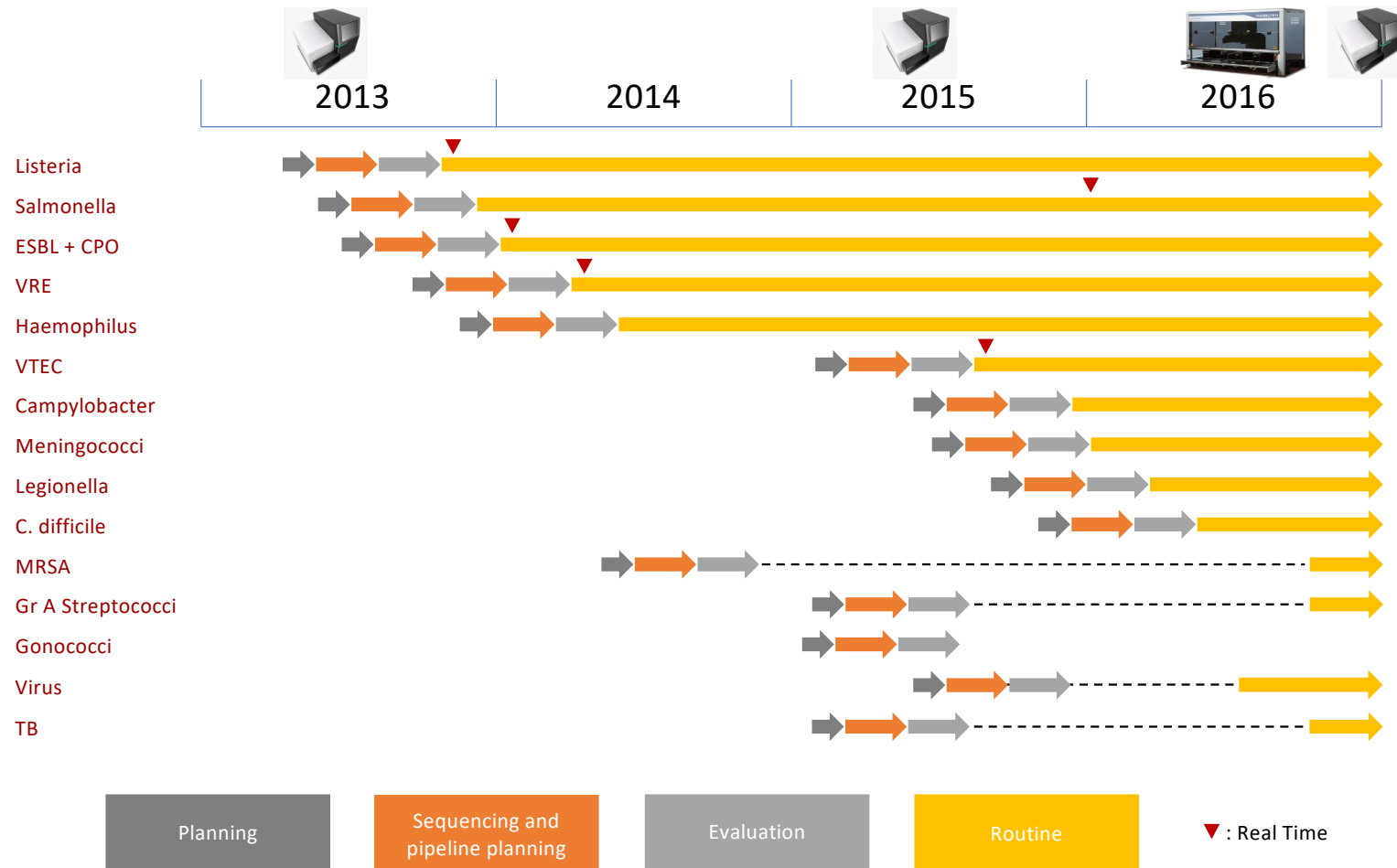
10-year “plan” in the making

How did we start working with NGS?

- Research was the facilitator
 - 2010 with 454 technology
- External providers
 - Small steps
- Collaborators!!!
- Students/PhD
- First Illumina instrument in 2013
 - Plug & Play solution



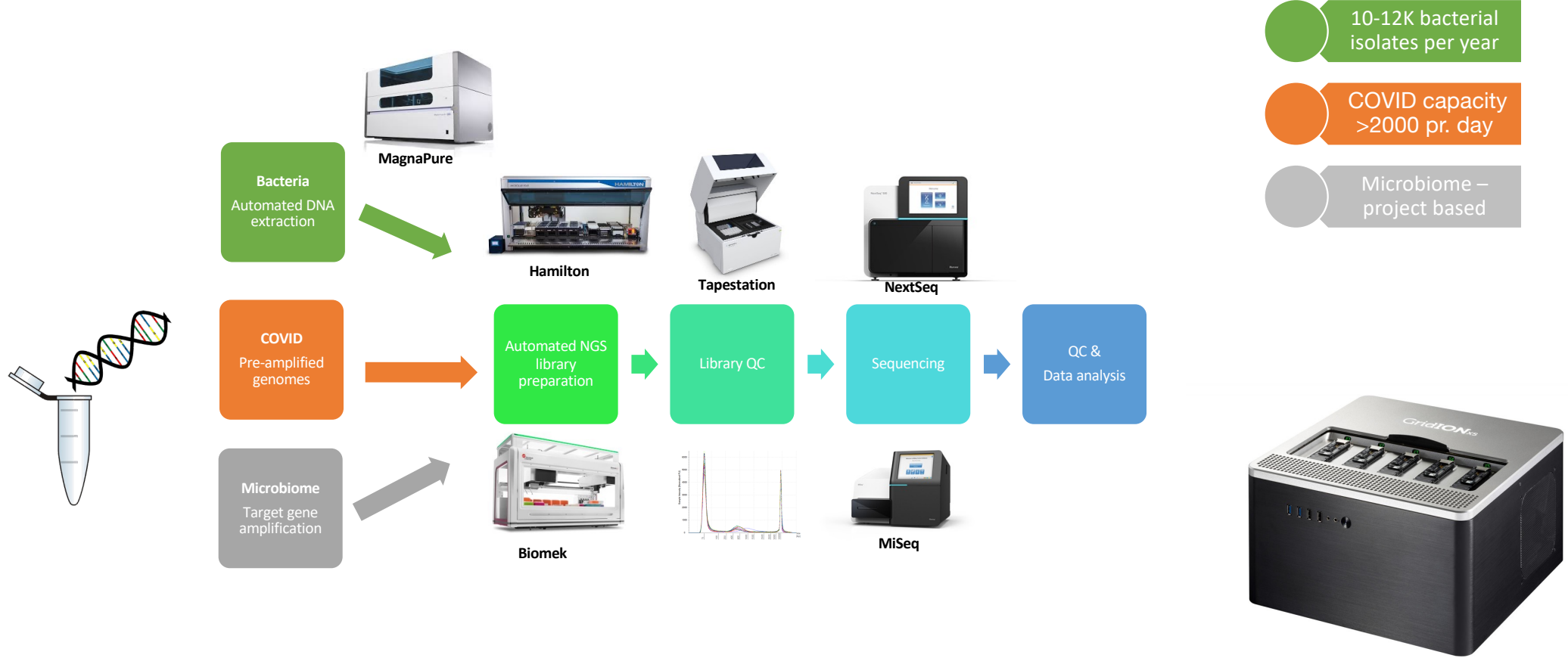
NGS at Statens Serum Institut





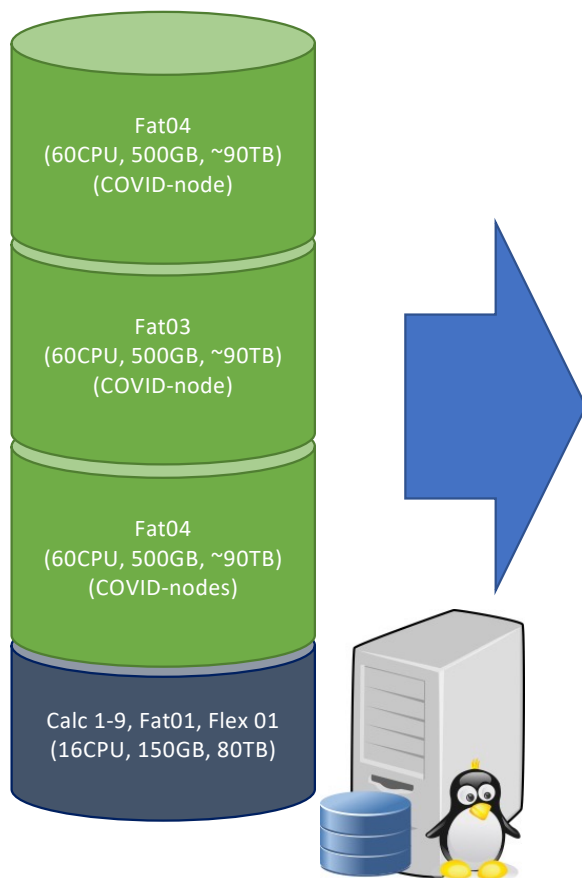
What is hiding in the sequences?

Sequencing Core Facility – From Sample to Sequence



HPC for bioinformatics analysis

- **Calc** system to be replaced by:



- **uGerm** on Computerome

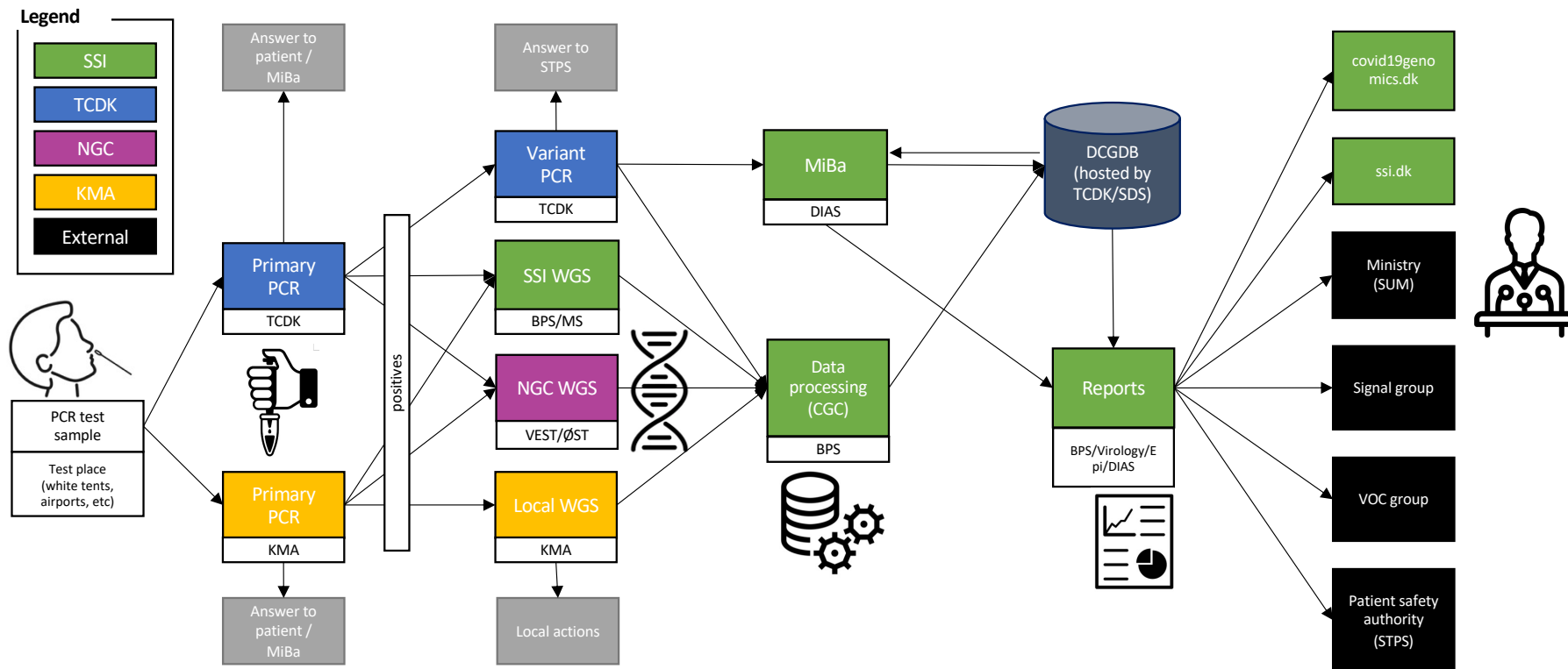


- Private cloud on computerome
- Scalable computing power (CPU)
- Scalable memory (RAM)
- Scalable storage (project allocated)
- GPU node for fast computation
- Computerome security clearance



- **Planned to be ready this year**

SARS-CoV-2 sequencing



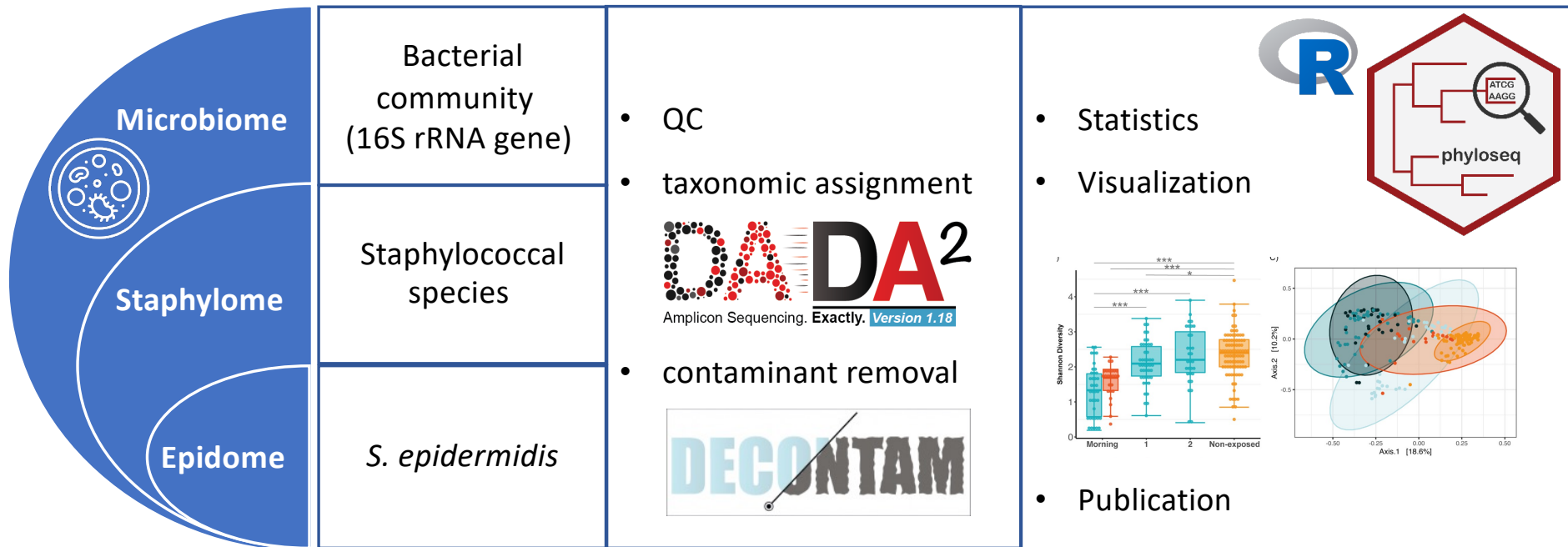
Total genomes sequenced: ~1 million samples

Very rapid turnaround time

Country / territory	Sequences shared	Reported COVID-19 cases	Reported deaths from COVID-19	% of cases sequenced and shared
Denmark	562,648	3,273,997	6,934	17.185
Luxembourg	43,965	287,100	1,123	15.313
United Kingdom	2,805,125	23,522,244	188,242	11.925
Canada	422,715	4,158,491	43,797	10.165
Sweden	210,322	2,569,152	19,904	8.186

Microbiome

Amplicon sequencing for research projects



Key aspect of WGS

QUALITY



Bacterial genomics

From core facility services to cutting edge research

- Providing software solutions to BPS and SSI:

bifrost Report

[Try out the new reporter \(beta\)](#)

[Wiki](#)

▶ Latest changes...

Run Name	220902_NB551234_0532_N_WGS_600_AHTC5WAFX3
Run Path	/s-sdi-calc1-p\data/BIG/bifrost/2022/220902_NB551234_0532_N_WGS_600_AHTC5WAFX3
Run Checker	http://s-sdi-calc2-p.ssi.ad:8051/220902_NB551234_0532_N_WGS_600_AHTC5WAFX3

Filter

220902_NB551234_0532_N_WGS_600_AHTC5WAFX3 (91) GO TO RUN

Supplying lab: Species: Provided Detected

Passed ssi_stamper: All values selected

APPLY FILTER (CLICK TO RELOAD)

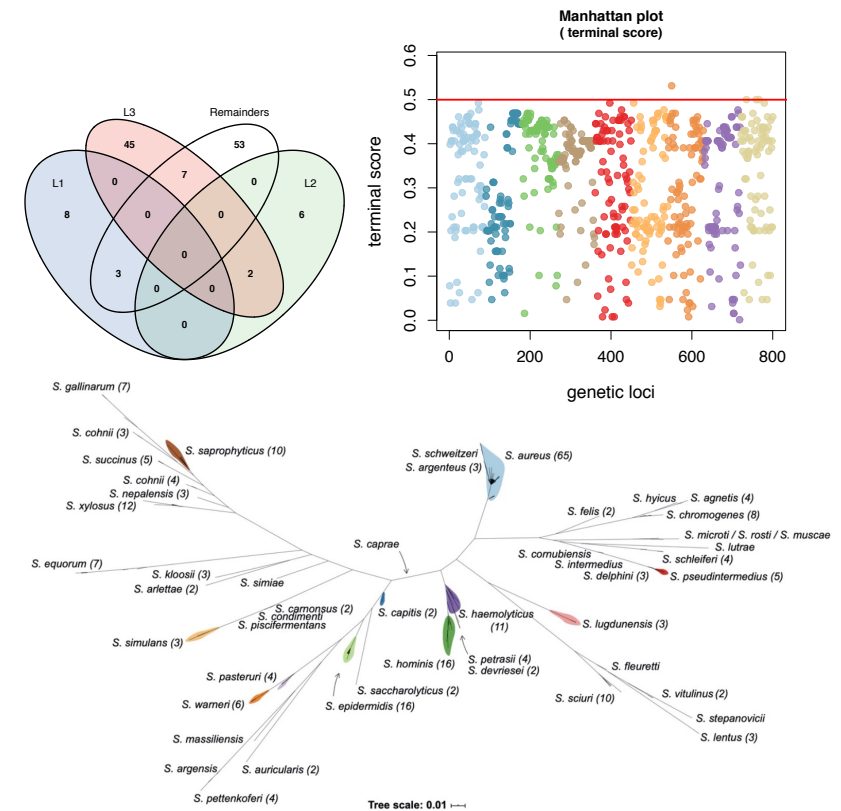
Campylobacter jejuni

2208F57772	
Sample Sheet	
Supplied name	2208F57772
User Comments	
Supplying lab	FBI
Submitter emails	
Provided species	<i>C. jejuni</i>
Read file	2208F57772_S64_L555_R1_001.fastq.gz
Detected Organisms	
<i>Campylobacter jejuni</i> + Unclassified	98.92%
<i>Campylobacter jejuni</i>	92.83%
<i>Campylobacter coli</i>	0.94%
Unclassified	6.08%
QC stamps	
ssi_stamper	pass:OK
▶ ResFinder/PlasmidFinder/VirulenceFinder/MLST (click to show)	
Supplying Lab Feedback: <input type="radio"/> Accept <input type="radio"/> Resequencing <input type="radio"/> Other <input checked="" type="radio"/> No action	
Assemblatron Results	
Number of filtered reads	3,424,740
Number of contigs (1x cov.)	86
Number of contigs (10x cov.)	86
NSI	131,907
Average coverage (1x)	272.46
Genome size at 1x depth	1,721,337
Genome size at 10x depth	1,721,337
Genome size 1x - 10x diff	0
Genome size at 25x depth	1,721,337
Ambiguous sites	1,639
MLST type: Novel	
Failed QC tests	
No failed tests.	

Bacterial species sequenced (2020 – 2022)

Species	No. Sequenced
<i>C. difficile</i>	3,311
<i>E. coli</i>	3,270
<i>E. faecium</i>	3,147
<i>C. jejuni</i>	2,996
<i>S. aureus</i>	2,926
Salmonella	2,388
<i>S. dysgalactiae</i>	1,665
<i>S. pneumoniae</i>	1,473
<i>S. agalactiae</i>	738
<i>L. pneumophila</i>	663
<i>K. pneumoniae</i>	446
<i>S. epidermidis</i>	382
<i>A. pleuropneumoniae</i>	320
Listeria	298
Gr. A. Streptococcus	276
<i>C. freundii</i>	255
<i>H. influenzae</i>	229
Yersinia	223
<i>C. perfringens</i>	137
Cronobacter	122

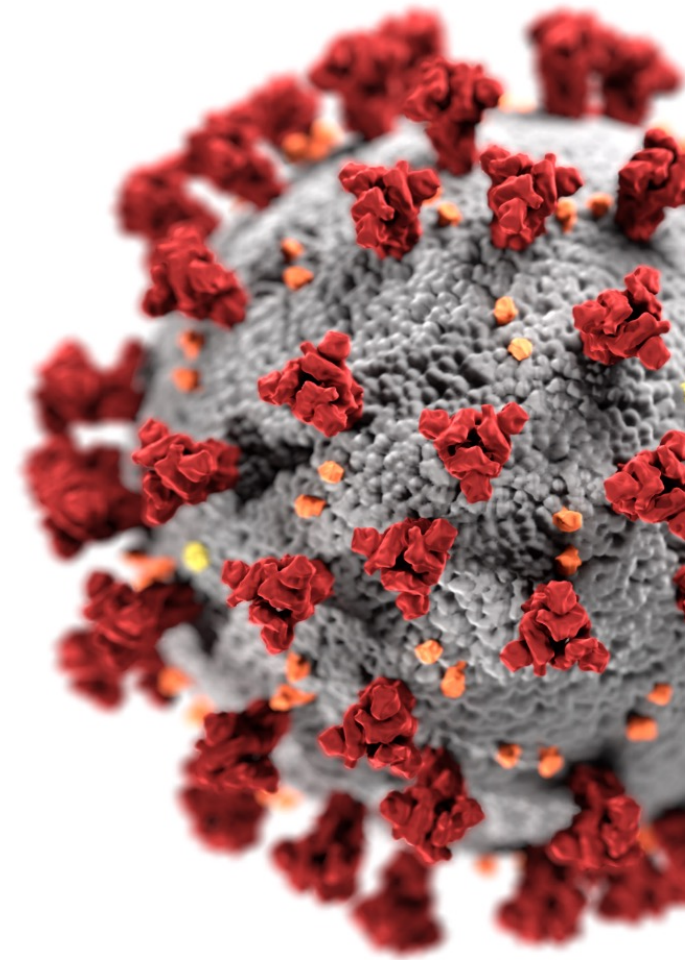
- Using data for research:





SARS-CoV-2 sequencing

A key for public health response

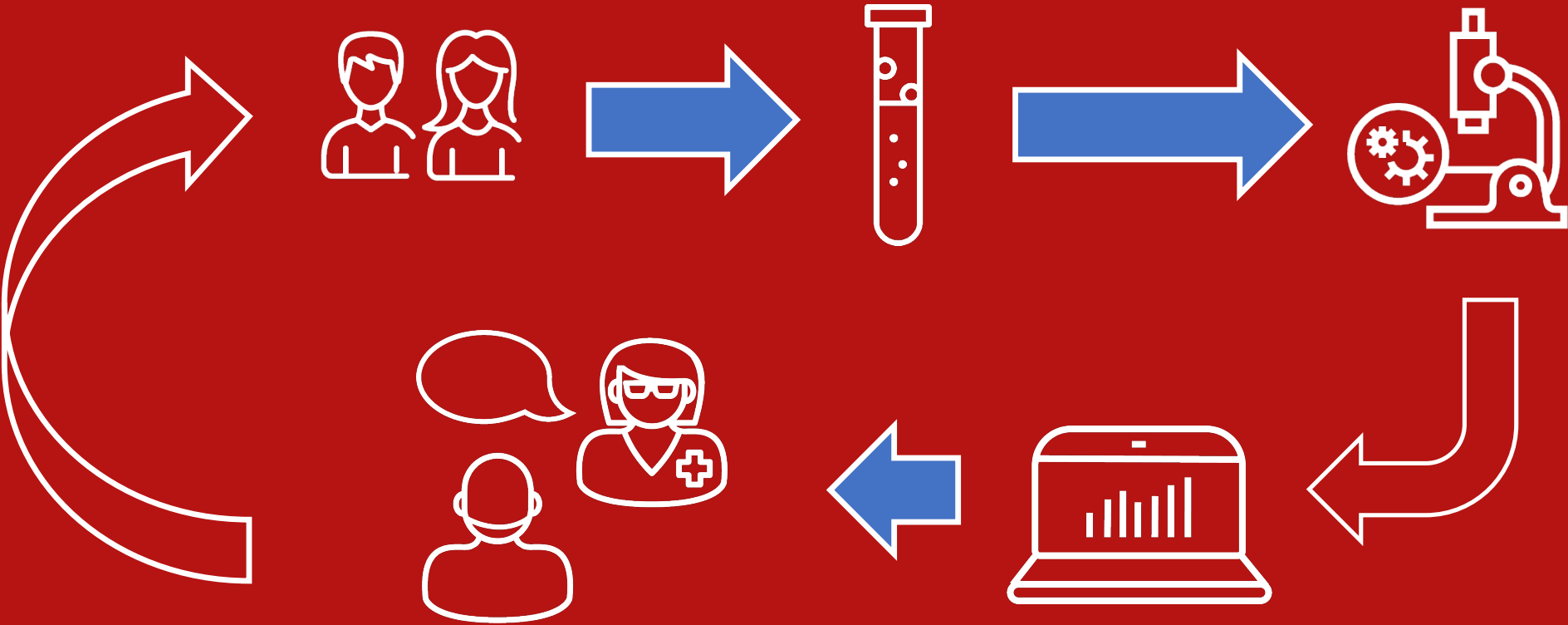


Denmark during the pandemic

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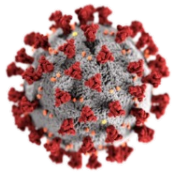
From individuals to surveillance and back



TestCenter Denmark



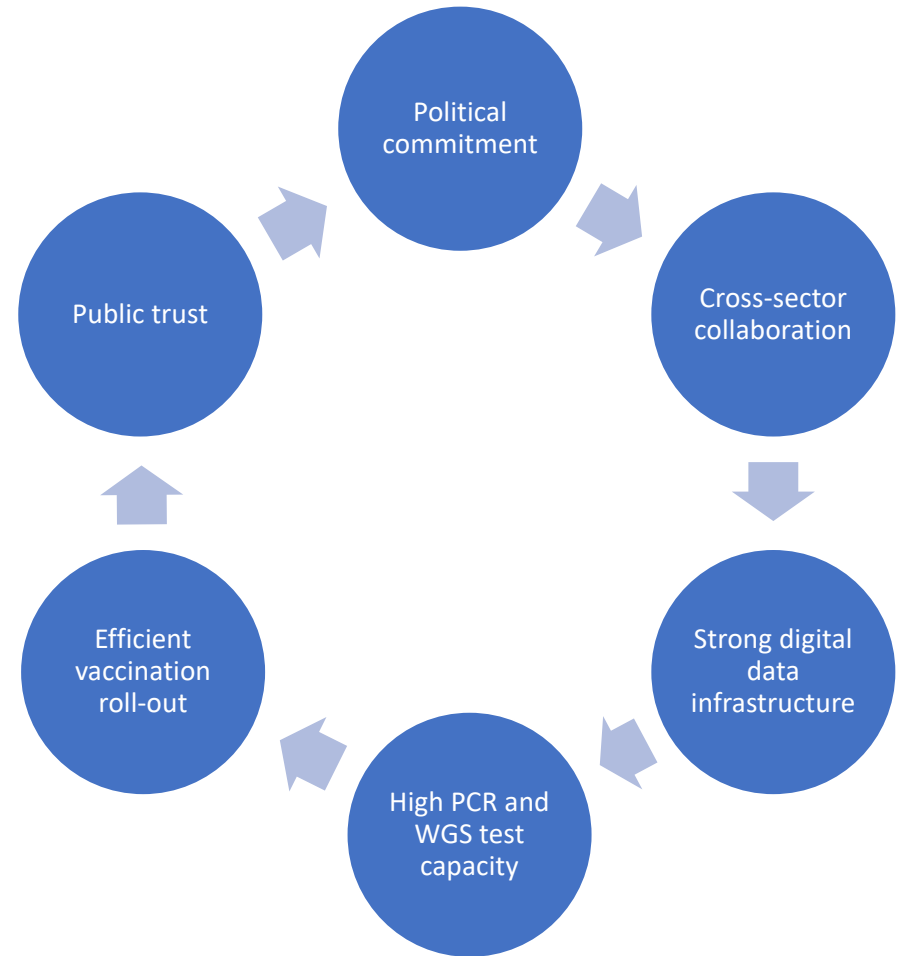
- Test Center Denmark-PCR community test track



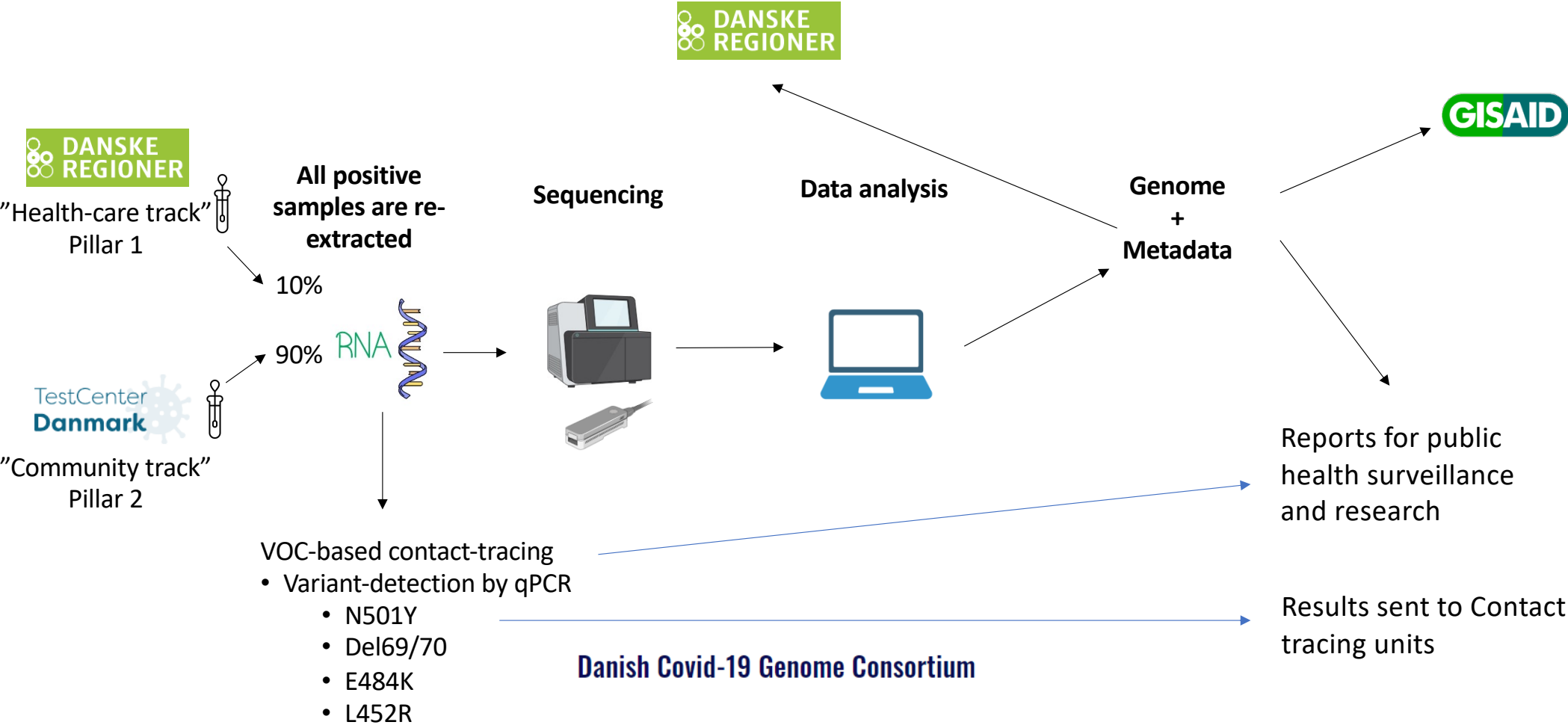
- The Danish COVID-19 Genome Consortium



- Use of WGS data for public health action

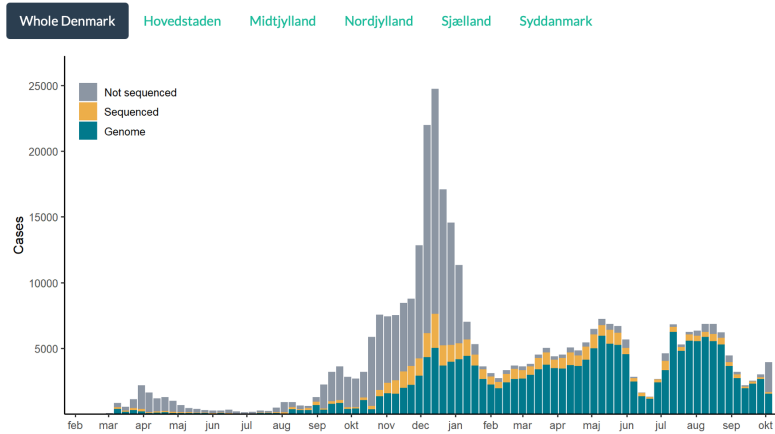


From Individuals to Data



Genomic overview of SARS-CoV-2 in Denmark

Danish Covid-19 Genome Consortium
Updated 2021-10-12



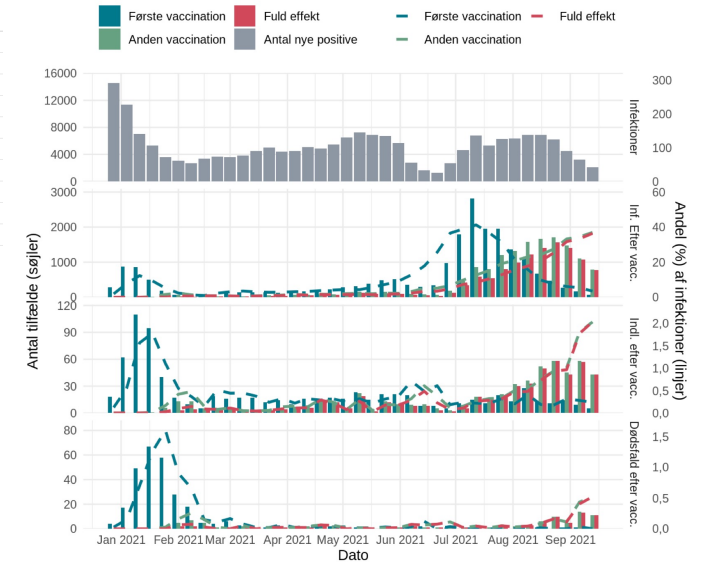
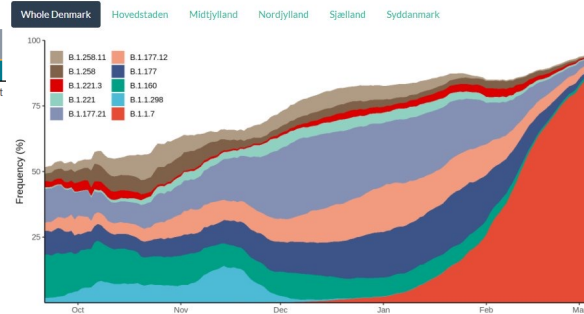
Genome coverage - table

The number of cases with a genome of sufficient quality relative to the total number of cases separated by "7" for each week (ISO standard) and region. The column **Denmark (Sequenced)** is the total number of cases sequenced including those that failed to produce a genome of sufficient quality.

Week	Hovedstaden	Midtjylland	Nordjylland	Sjælland	Syddanmark	Denmark (Genomes)	Denmark (Sequenced)
2021-W40	865/1963	145/560	75/216	235/531	209/827	1542/3957 (39%)	1685/3957 (42.6%)
2021-W39	1378/1580	344/356	177/196	359/409	378/437	2653/3012 (88.1%)	2779/3012 (92.3%)
2021-W38	937/1029	322/344	289/288	367/400	371/412	2299/2524 (91.1%)	2477/2524 (98.1%)
2021-W37	918/1024	330/348	143/153	242/273	322/350	1965/2173 (90.4%)	2095/2173 (96.4%)
2021-W36	1382/1641	438/494	123/143	432/501	347/402	2738/3211 (85.3%)	3002/3211 (93.5%)
2021-W35	2126/2331	501/738	138/248	407/457	446/634	3650/4455 (81.9%)	3943/4455 (88.5%)
2021-W34	2838/3185	871/1081	274/366	488/563	795/971	5298/6206 (85.4%)	5799/6206 (93.4%)
2021-W33	2565/3051	1083/1397	444/582	495/593	910/1164	5538/6853 (80.8%)	6087/6853 (88.8%)
2021-W32	2275/2734	1420/1578	620/690	603/733	906/1063	5865/6858 (85.5%)	6253/6858 (91.2%)
2021-W31	2154/2456	1312/1495	667/744	460/543	911/1058	5546/6351 (87.3%)	5958/6351 (93.8%)

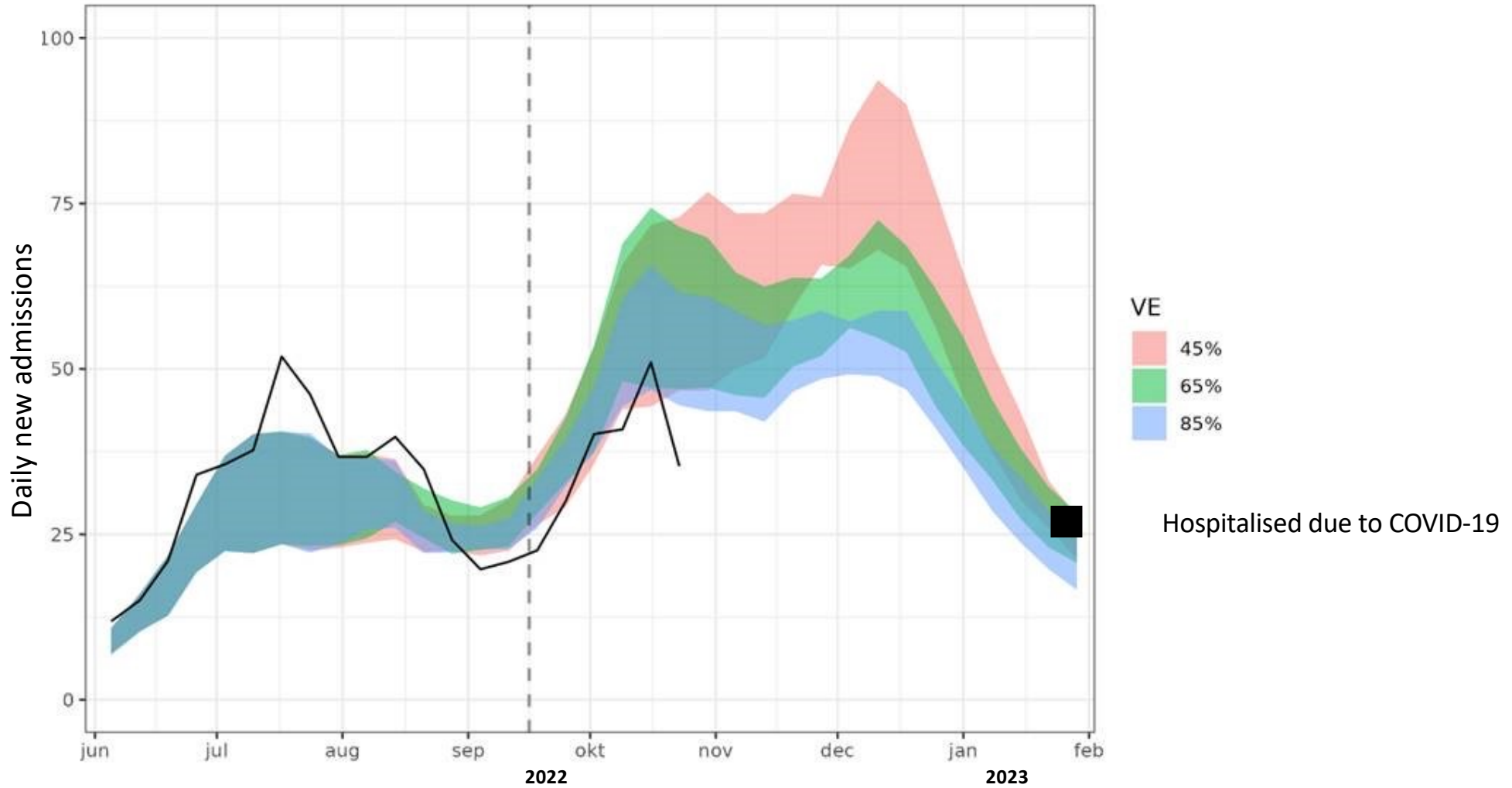
Dominating lineages

Frequency of the 10 most abundant PANGO lineages across Denmark within the last 6 months. The frequency is calculated as a centered 14-day rolling average. Note that some regions have low case-counts in some periods, which can make small absolute changes look dramatic on a relative scale.



Genomic data in relation to QC, lineage and variant assignments, outbreak investigations, breakthrough infections etc

Modelling of SARS-CoV-2



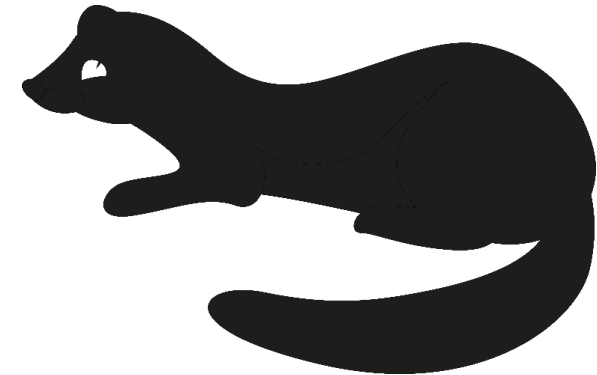
Animal Health Surveillance - Mink Outbreak June-November 2020

Occupational Risk

- ~3,500 people living on mink farms in Denmark (19%)
- ~400 migrant workers on 8 pelting facilities

Community Spread of B.1.1.298

- Estimated 5,000 cases
- Primarily local community spread in regions with SARS-CoV-2 in mink



Animal Health Surveillance - Mink

Spike mutation frequency

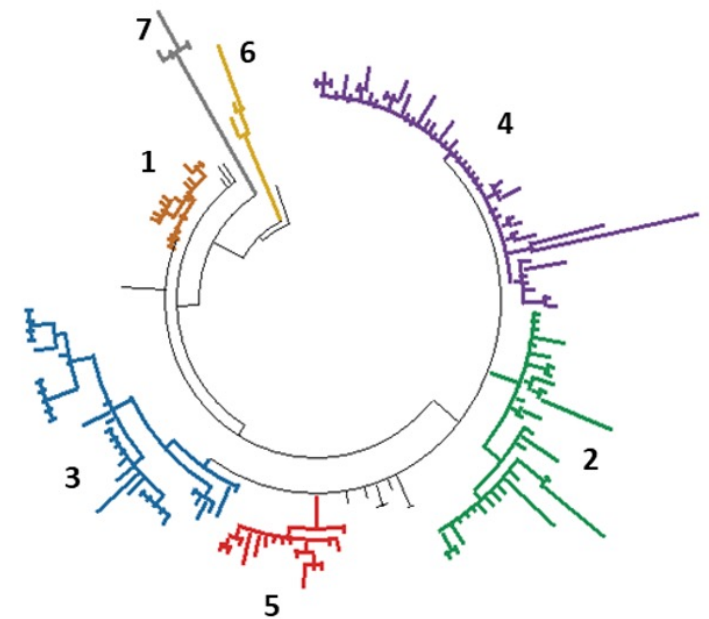
Transmission cluster	Spike mutations co-occurring*	Frequency in humans**
1	453F	N = 142
2, 3, 4	69-70delHV, 453F	N = 162
4	69-70delHV, 453F, 1147L	N = 18
5	69-70delHV, 453F, 692V, 1229I	N = 12

* All SARS-CoV-2 mink-associated sequences also contained the D614G

** Up until 30 October 2020

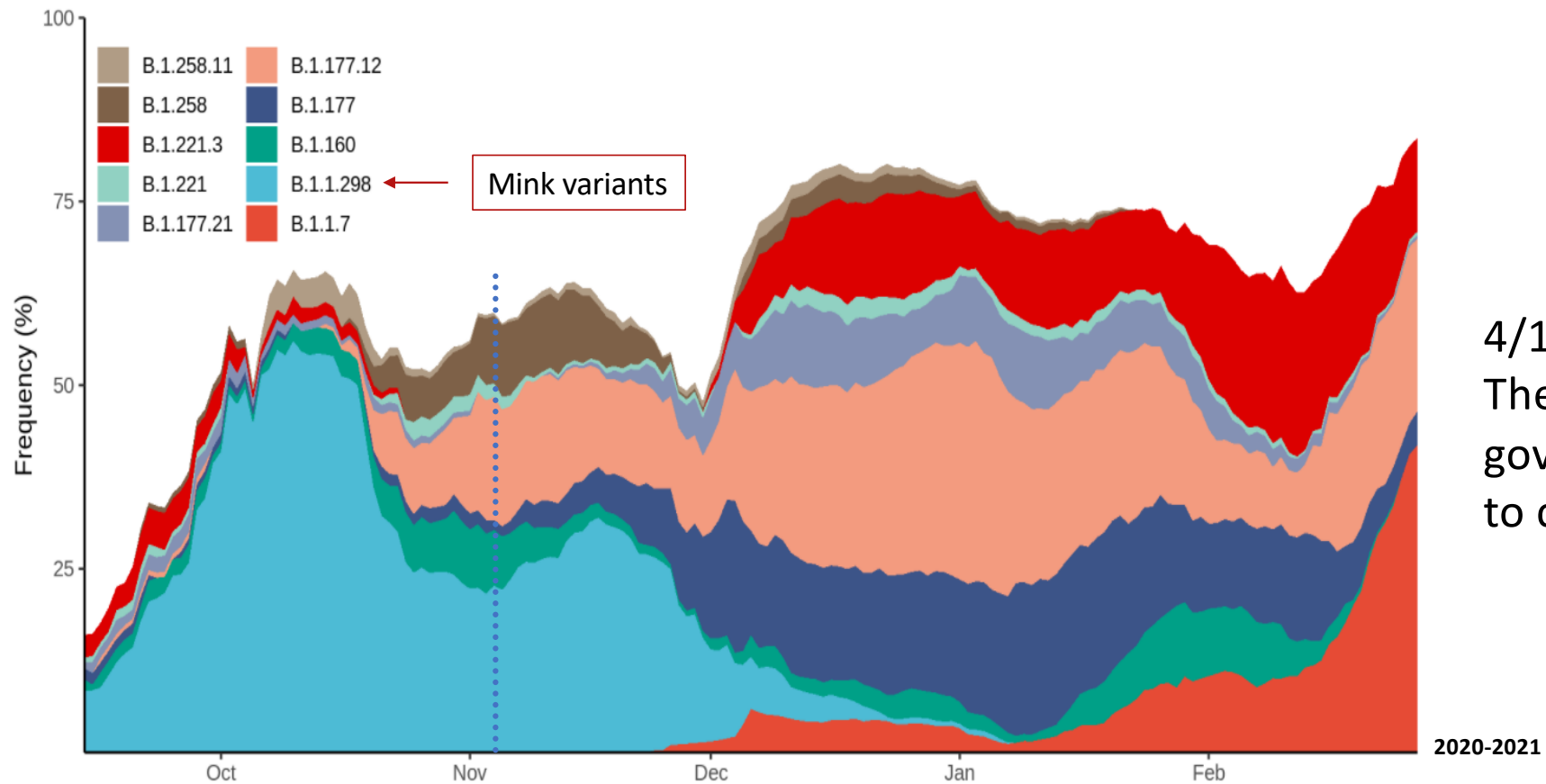
- Cluster 5 had 4 spike mutations (at this time this was unusual)
- Reduced sensitivity to neutralizing antibodies

Transmission Cluster



Animal Health Surveillance - Mink

Community spread of mink variants, North Denmark



4/11 2020:
The Danish
government decided
to cull all mink

Other important sequenced-related outcome

ARTICLE

<https://doi.org/10.1038/s41467-021-27202-x>

OPEN

Increased transmissibility of SARS-CoV-2 lineage B.1.1.7 by age and viral load



RESEARCH ARTICLE

Vaccine effectiveness against SARS-CoV-2 infection or COVID-19 hospitalization with the Alpha, Delta, or Omicron SARS-CoV-2 variant: A nationwide Danish cohort study

ARTICLE

<https://doi.org/10.1038/s41467-022-31494-y>

OPEN

Effect of vaccination on household transmission of SARS-CoV-2 Delta variant of concern

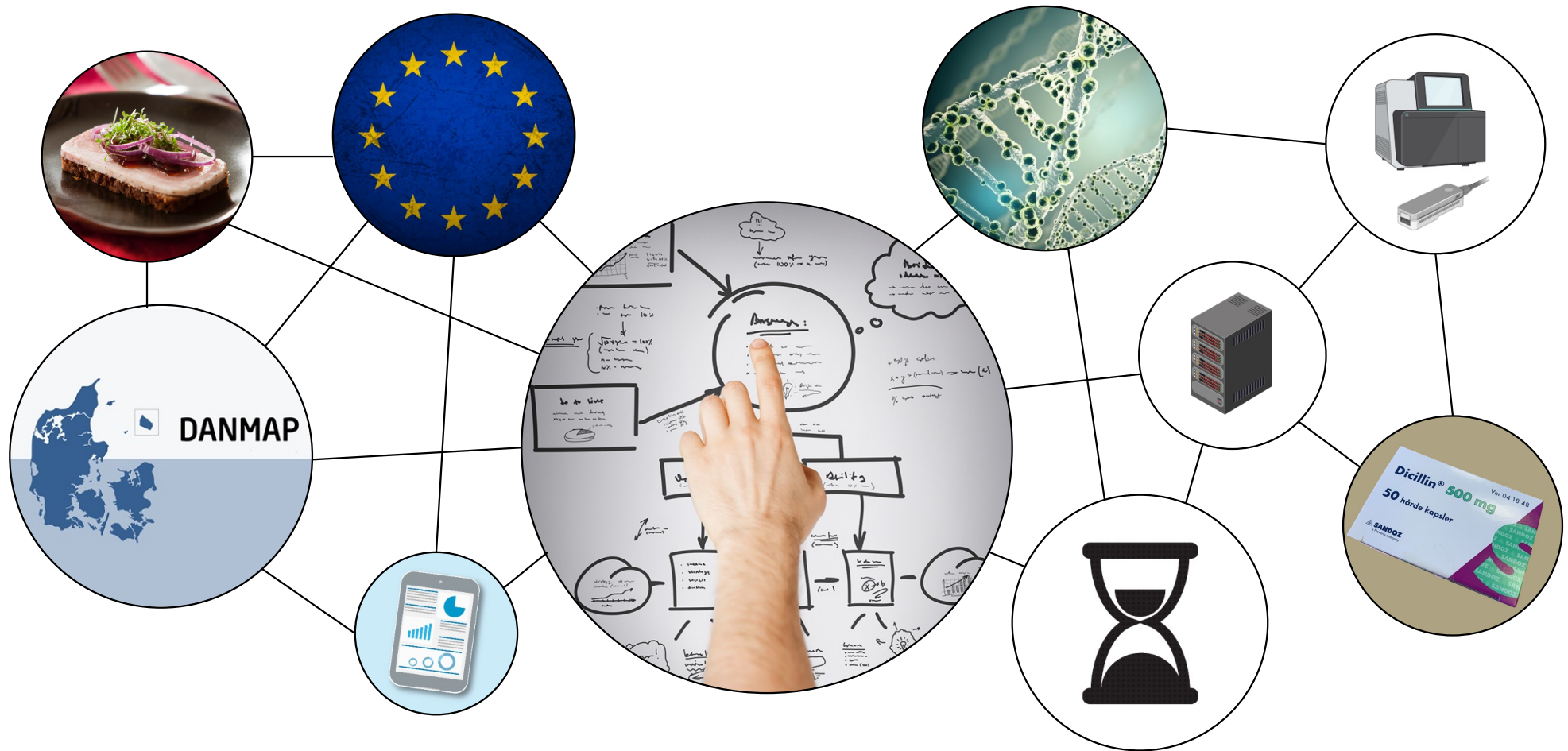


Risk of hospitalisation associated with infection with SARS-CoV-2 omicron variant versus delta variant in Denmark: an observational cohort study

Occurrence and significance of Omicron BA.1 infection followed by BA.2 reinfection

Risk of reinfection, vaccine protection, and severity of infection with the BA.5 omicron subvariant: a nation-wide population-based study in Denmark

Other examples of usage of WGS



Contact tracing via WGS og LPR



Upload data file (xlsx):

Browse...

No file selected

Department

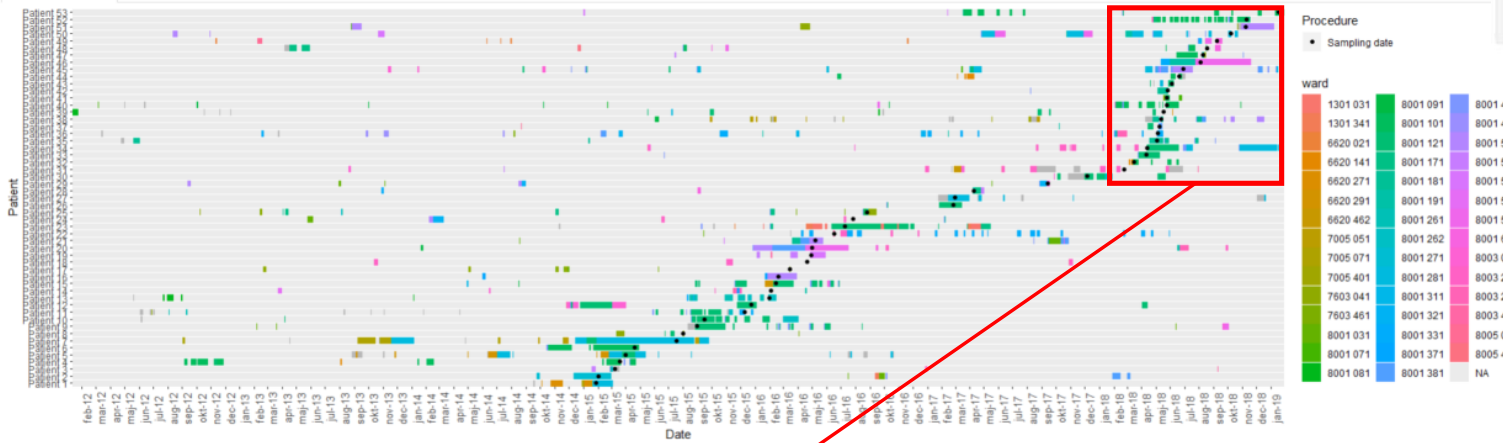
Department

Hospital

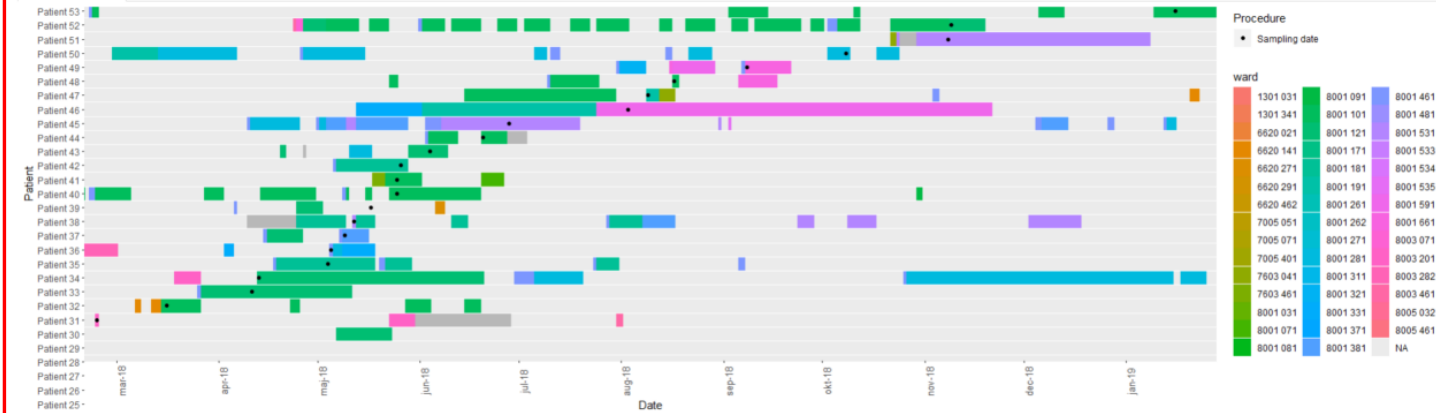
Buffer time

Infection loads

Demographics



Department Hospital Buffer time Infection loads Demographics



Group A Streptococcus in Denmark

Genomic and epidemiological trends in
the post-lockdown period



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Epidemiology of Group A Streptococcus

- Group A Streptococcus (*Streptococcus pyogenes*) can express a diverse range of virulence factors and cause a wide spectrum of both superficial (GAS) and invasive disease (iGAS)
- There is no clinical reporting system for GAS or iGAS in Denmark
- Current surveillance efforts are instead based on extracting positive test results from the national Microbiology Database (MiBa), in combination with a number of national registries to collect patient characteristics

Strep throat



Scarlet fever



Necrotizing fasciitis



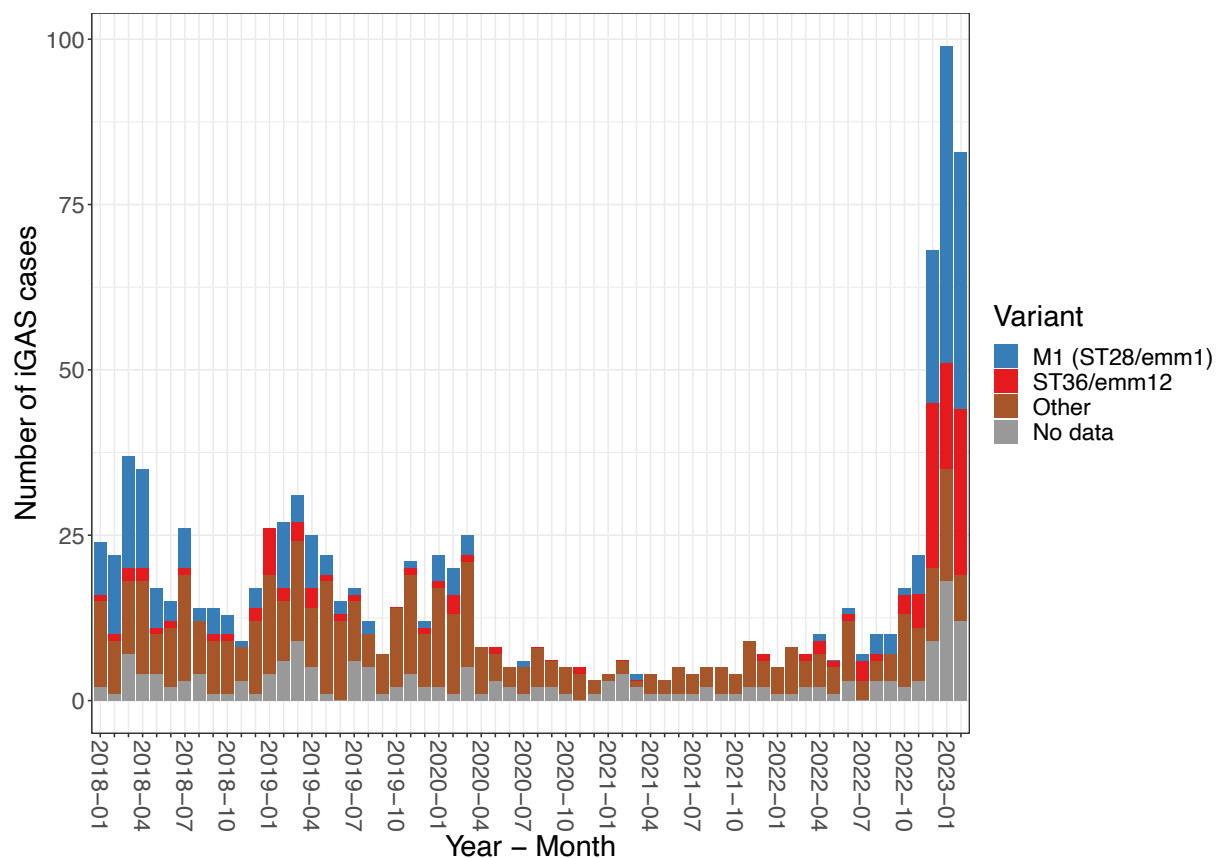
Genomic trends

Clinical microbiology laboratories submit iGAS isolates to Statens Serum Institut for characterization on a voluntary basis

Thanks to this effort, whole-genome sequencing data is available for 85% of iGAS cases since 2018.

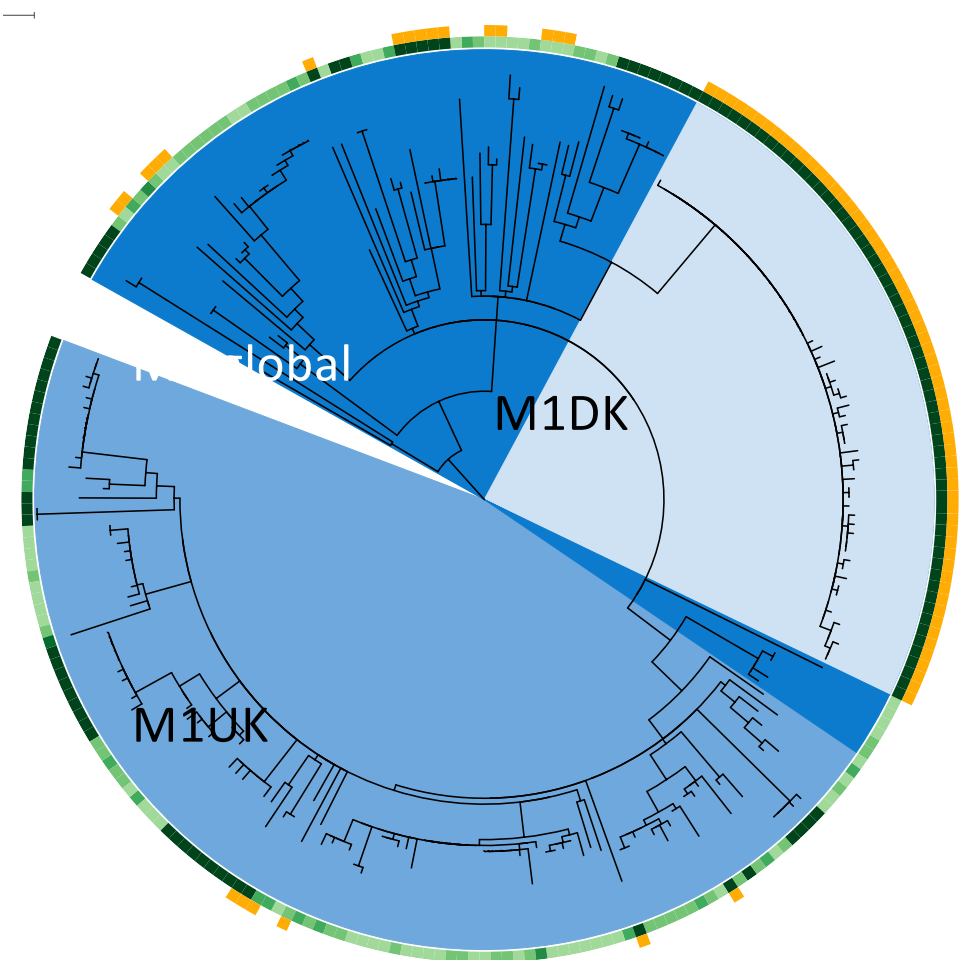
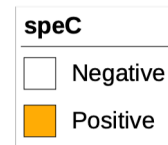
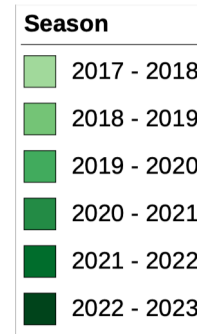
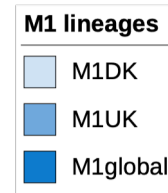
In addition, laboratories submitted 257 GAS isolates from specimens obtained by general practitioners from patients with non-invasive infections in January and February 2023.

Variants in iGAS samples since 2018



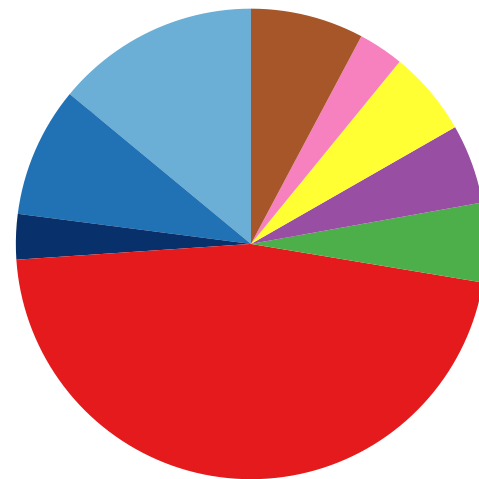
Characteristics of M1 in Danish iGAS

- >50% of iGAS cases in 2023 were caused by the M1 variant (ST28/emm1.0)
- Novel M1-lineage has spread rapidly since its first appearance in late 2022 and has been responsible for 30% of iGAS cases in 2023.
- In addition to constituting a distinct phylogenetic clade, this variant is characterized by an acquisition of a bacteriophage containing the virulence gene *speC*

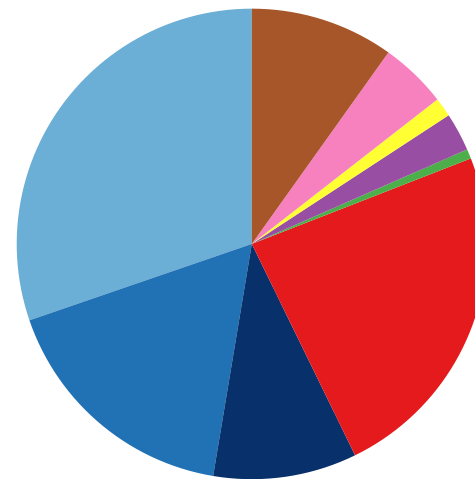


Core genome phylogeny of Danish M1 iGAS

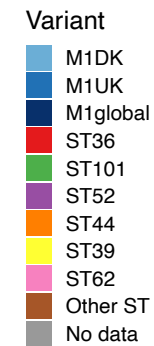
Variant distribution of GAS and iGAS in January-February 2023



Non-invasive isolates (n=257)

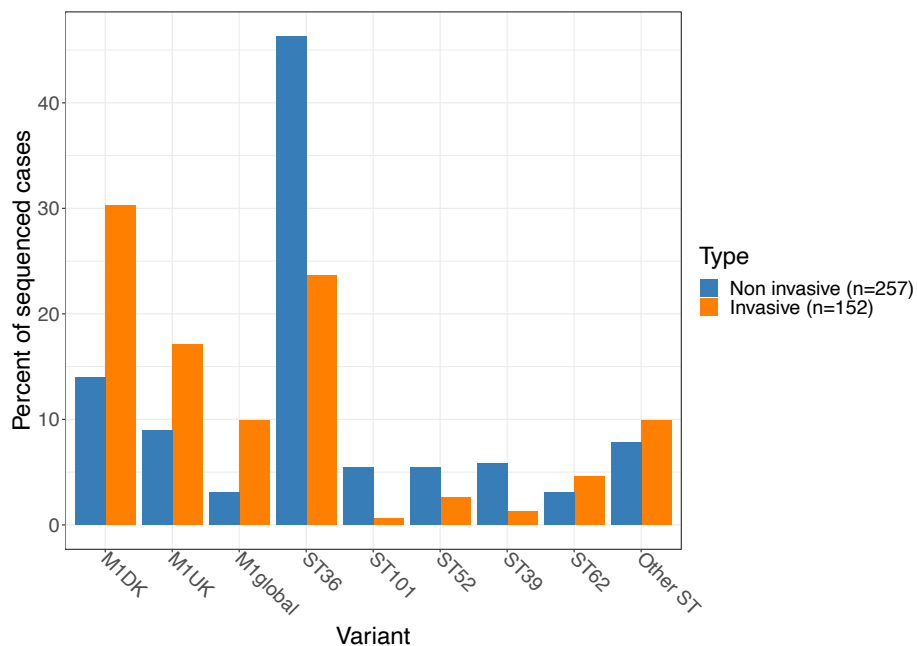


Invasive isolates (n=152)



Virulence of M1 variants

- M1 variants were significantly overrepresented in invasive cases relative to non-invasive
- No significant difference in iGAS case fatality rate between variants
- iGAS patients infected with M1 variants more often require intensive care treatment



Variant	iGAS cases	% Intensive care cases	p-value	OR
M1DK	62	38.7	<0.05	2.38 (1.32-4.2)
M1UK	119	31.9	<0.05	1.8 (1.14-2.8)
M1global	70	32.9	<0.05	1.8 (1.01-3.13)
ST36	111	21.6	NS	0.96 (0.56-1.58)
ST101	69	17.4	NS	0.72 (0.34-1.39)
ST52	62	19.4	NS	0.83 (0.39-1.62)
ST44	35	17.1	NS	0.71 (0.24-1.78)
ST39	34	2.9	<0.05	0.1 (0-0.61)
ST62	21	9.5	NS	0.36 (0.04-1.52)
Other ST	256	17.6	<0.05	0.66 (0.45-0.97)