It always seems impossible until it's done

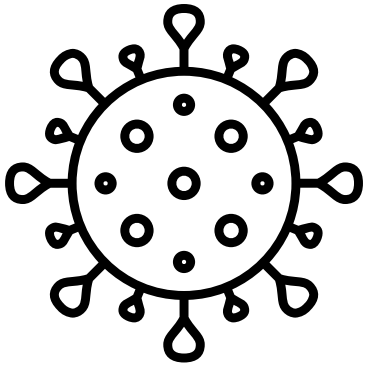
– Vårt bidrag till pandemiberedskap i stormens öga och vidare

Jessica J Alm

National Pandemic Center, NPC
CTMR, Department for Microbiology, Tumor and Cell Biology,
Karolinska Institutet

2022-08-29

The storm is coming



Mars 2020
- Corona hit Sweden

Forskningslabb stänger ner/
lock down

MARCH 2020

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

KUL behövde hjälp

KAROLINSKA
UNIVERSITETSLABORATORIET

The storm is coming – March 2020

Professor
Mathias Uhlén,
KTH

Professor
Lars Engstrand,
KI

Fredrik Boulund & Marica Hamsten

CTMR

Centre for Translation Microbiome Research, CTMR, MTC, KI

Activities focus on understanding the contribution of the human **microbiome** to physiology and pathophysiology

- Jan Albert kontaktade Lars Engstrand – kan CTMR hjälpa KUL men **RNA extraktion** för SARS-CoV-2 PCR diagnostik
- Brist på reagnes, stora behov globalt

Extractions for the hospital

KAROLINSKA UNIVERSITETSLABORATORIET

CTMR **Centre for Translation Microbiome Research, CTMR**

SP-960

- Vad mer kan vi göra för att hjälpa?
- Logistik för att hjälpa sjukhusets labb?
- Hur kan vi hjälpa nationellt?

CoV-2 extraction to assist KUL

- Deep well plate with inactivated samples (Roche buffer) from the hospital -> gave back extracted RNA
- No information about the samples
- About 8 plates a day (all night work) - Slow process!

Capacity >5000 samples/day

Sample reception and preparation

1. Sampling

2. Virus inactivation

3. Transfer to 96 deepwell plate

Inactivated samples in 96 deepwell plate

RNA extraction

4. RNA extraction

MGISP-960

Eluted RNA in PCR plate

qPCR analysis

5. qPCR

qPCR instrument

qPCR CT-values

Disseminate results

6. Report results

Database and web service

CTMR SciLifeLab Karolinska Institutet Knut och Alice Wallenbergs Stiftelse

Vad krävs för att bli ett diagnostiklabb?

- ✓ IVO ansökan
- ✓ KI inte tillstånd att göra diagnostik, massa diskussioner med Folkhälsomyndigheten och KI
  - -> Bemyndigande från regeringen
- ✓ Checklista från FoHM
- ✓ Mycket hjälp från KUL
  - Dokumentation
  - Träning
  - Praktiska aspekter
- ✓ Måste ha ett namn: *National Pandemic Center*

Inspektionen för vård och omsorg

Folkhälsomyndigheten

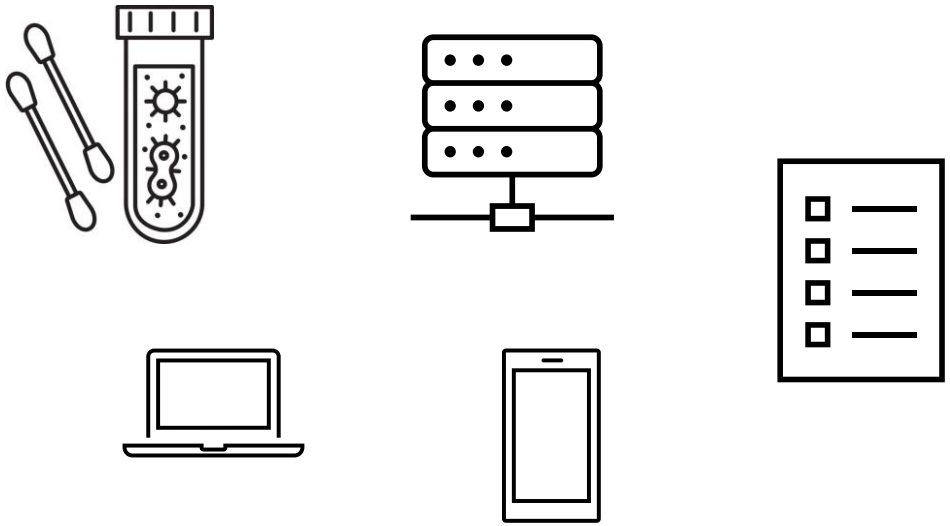
Regeringen

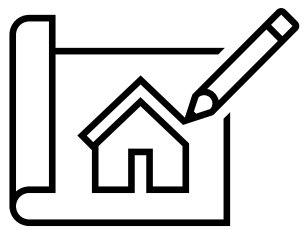
KAROLINSKA UNIVERSITETSSJUKHUSET

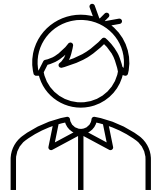
NPC

Setting up the lab

- Lab space
- Protocols and workflows
- Instruments
- Personnel
  - Key competences
- Laboratory Information Management System (LIMS - not Excel)
- Sample registration and result





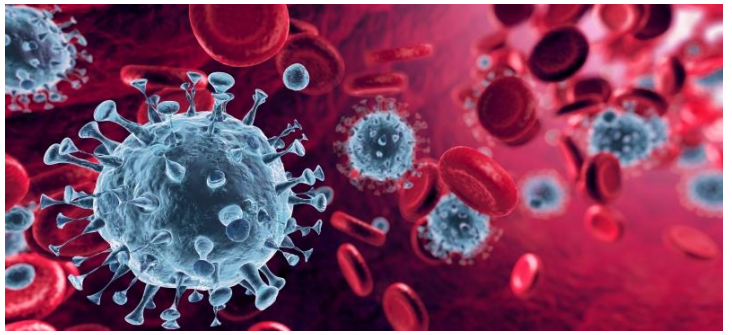


Wallenberg donation, 40Msek

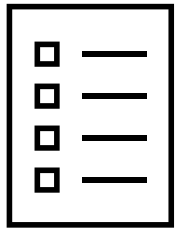
- SAS flight: Hong Kong – Stockholm
  - Instruments (5 extraction robots)
  - Extraction reagents
  - PCR reagents
  - Sampling materials
  - Plastics
- Lab equipment
- Salaries

- KI erbjöd tillgängliga utrymmen i Biomedicum
  - Hyresfritt
  - Labbet växte över flera våningsplan och institutioner

Protocols



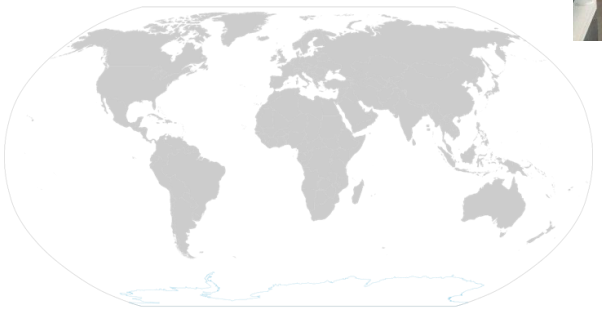
- Inactivation - a struggle
- Extraction protocol in place
  - Set up more robots
- QPCR protocol relatively easy to set up
  - Finding instruments was hard
- Workflow and throughput
  - Have samples in all steps at all times



→ **How to build a diagnostic pipeline**

Inactivation Protocol: How to stay safe in the lab

- Inactivation - Not efficient adding a buffer
  - Time consuming
  - Risk of being exposed to the virus
  - Dependent on access to inactivation buffer



- What method to use?
  - KUL, FoHM, Örebro, France, Germany
  - Heat inactivation was chosen

Sample reception and inactivation

- BSL2+
- Only trained personnel
- Strict routines

Checklist – How to enter and leave the virus lab

IN

Outer corridor	1. Inner disposable coat	8. Inner disposable coat
Fore room/ Zone 1	2. Face mask 3. Eye protection 4. Inner/clean gloves	7. Face mask 6. Eye protection 5. Inner/clean gloves
Clean are = inside taped area/ Zone 2	5. Autoclavable shoes 6. Autoclavable outer lab coat	4. Autoclavable shoes 3. Autoclavable outer lab coat
	Enter	Exit
Inactivation lab/Zone 3	7. Outer ("dirty") gloves	2. Remove outer ("dirty") gloves 1. Spray hands with 70% ethanol

OUT

- Validated protocol for heat inactivation:
  - Ambient temp 75C
  - Samples kept in oven for 45 min

To guarantee that:

⇒ the liquid in all tubes reach the critical temp of 56C

⇒ for the critical period of time

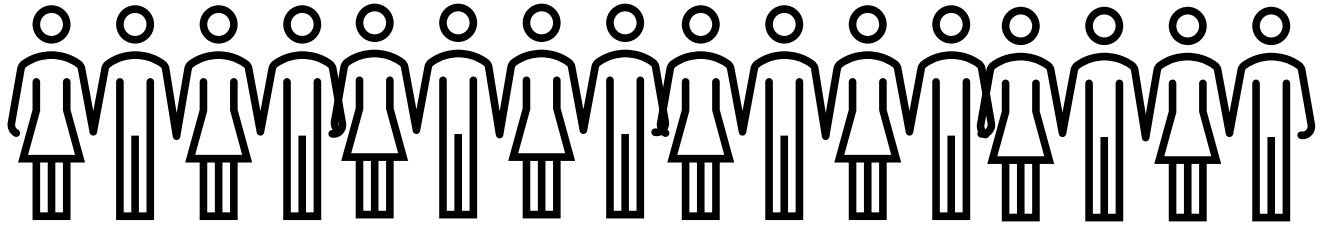
⇒ to ensure proper inactivation of the virus

Instruments

- Worldwide lack of equipment to buy
  - Sample pipetting robot
  - qPCRs
  - UV benches
  - LAF benches
- Robots from BGI, Tecan, and Hamilton
- qPCR borrowed from other groups at KI
- Borrowed 30 freezers and fridges, and 5 LAF-benches from BioClinicum

Personnel

- CTMR personnel
- People offered their skills from other research groups at KI, KTH, SU, etc.
- Finding key persons



How to keep track of everything
NPC needed a LIMS

Fredrik Boulund
Head of IT and Bioinformatics

NPC

Laboratory Information Management System (LIMS)

- Digital system to track samples through the analysis process
- **Ensure correctness and traceability**
- **Records important information**, e.g.:

  Sample barcode
  Origin of sample
  Date and time of processing in each step

- **Communication with external systems (**Direkttest, SmiNet)
- **Statistics and quality control**

BaseSpace CLARITY LIMS

DirektTest SARS-CoV-2

SmiNet
Elektronisk anmälan av smittsamma sjukdomar

- A new LIMS was built up - Made possibly by the help of 4 developers from SNP&SEQ Technology Platform at SciLifeLab Uppsala
- 8 contributors in total - About 18,000 lines of code
- Open-source implementation under MIT-license

Clarity LIMS™
Intuitive Lab Management™

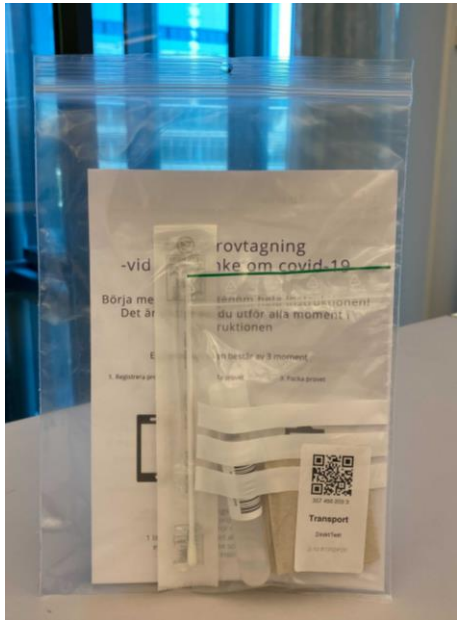
Sample registration and result

DirektTest SARS-CoV-2

- Experienced with large-scale testing
  - Previously involved with Chlamydia self-testing in Västra Götaland
  - Already had IT-systems for sample registration and result communication in place
- Register persons to samples via BankID
  - NPC keep no personal information in our lab systems
- Sends SMS to notify when test result is available

Up and running på 4 veckor!

Shortage of test kits in early May



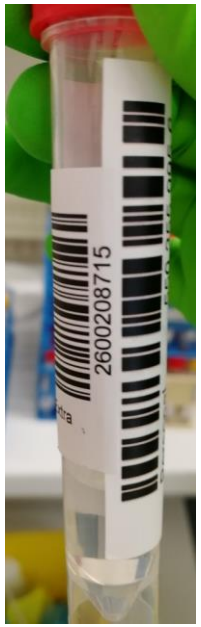
- Global shortage of all kit components
  - Tubes
  - Swab
  - Buffer fluid
  - Instructions
  - Plastic bags

- We decided to implement kit packing at NPC together with Direkttest
  - NPC takes control of the logistics chain to ensure reliable deliveries of kit components

Logistics
Labels
Control

Kitpacking

- Kit packing is challenging:
  - Ensuring correct label placement on test tubes (hard lessons)
  - Ensuring there are no duplicated barcodes
  - Staff intensive, one person can pack approx. 350-400 kits per day
  - Logistics



VÄSTRA GÖTALANDSREGIONEN

- Pioneer region
  - Established connection with direkttest.se and analysis at Sahlgrenska university hospital
- **April:**
  - Tent outside the hospital for staff
  - Symptomatic hospital personnel at home
- Sahlgrenska lab capacity not high enough
  - Daily transports from Västra Götaland to NPC at KI
  - New test kits returned with the same transport every day

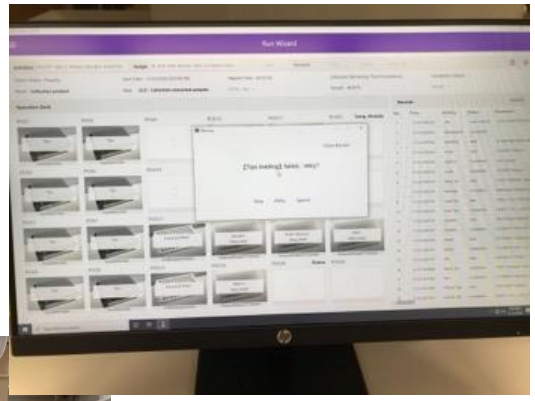
Region Stockholm

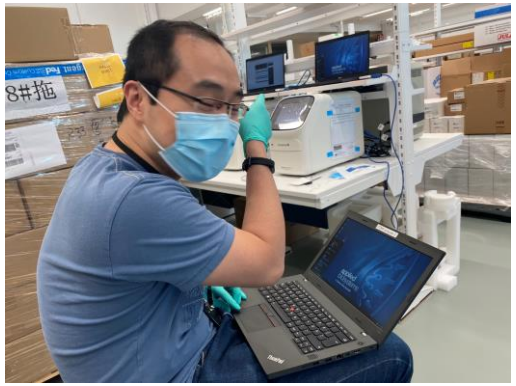
- **Mid-April:** Research study by Karolinska hospital
  - Testing healthcare workers
- **End of April**
  - SLL Healthcare workers allowed testing via Alltid Öppet
- **Mid-June**
  - **General public via Alltid Öppet**

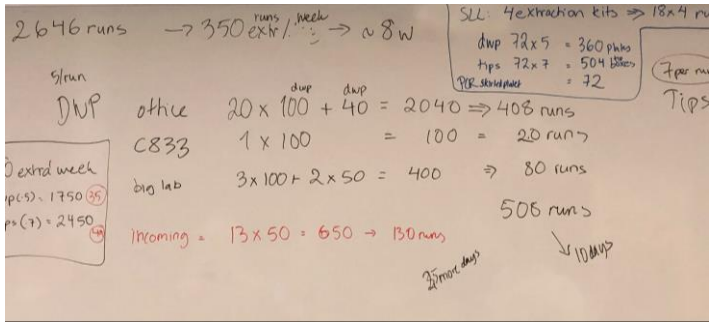
region västerbotten

- **June**
  - Samples to NPC
  - All categories
- Daily transport by car from Umeå
- September
  - Study at Umeå University

Get shit done



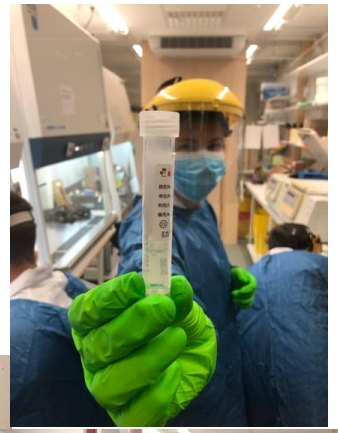


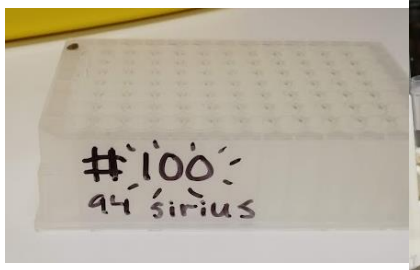


NPC Shift Leader 2:46 PM
Estimated total number of plates today is 103

November 26th, 2020

NPC Shift Leader 11:16 AM
Estimated total number of plates today is 96
1





VÄSTRA GÖTALANDSREGIONEN

region västerbotten

Region Stockholm

24h

- 9 månader
- 3 regioner
- Massvis med material och reagenser

- 231 anställda
- 3 shiftesarbete
- 47 800h månadsanställda och 28 800h timanställda
- 10 200h OB, 4 300h övertid

- 143,193 meddelanden i slack (1 per minut)

- **Engagemang och målmedvetenhet**
- **Teamwork**

National Pandemic Centre SARS-CoV-2 (COVID-19) test statistics

⚠The National Pandemic Center (NPC) at Karolinska Institute ceased operations of high throughput PCR diagnostics on 2020-12-21.

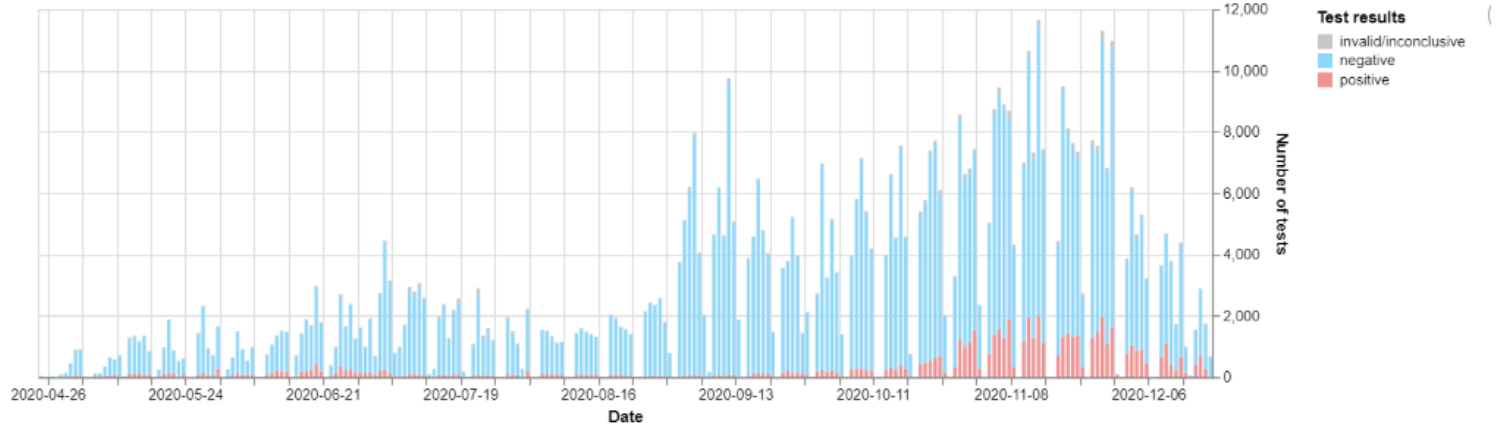
The data presented here receives no further updates and only remains for historical reference.

KI Press Release

NPC test numbers

The number of SARS-CoV-2 (COVID-19) tests run daily or weekly, split up into positive, negative and invalid/inconclusive results.

Daily



Sum total

621783

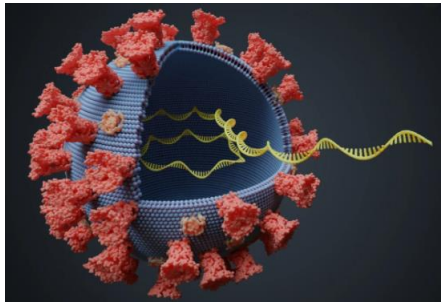
December 2020

NPC DIAGNOSTICS

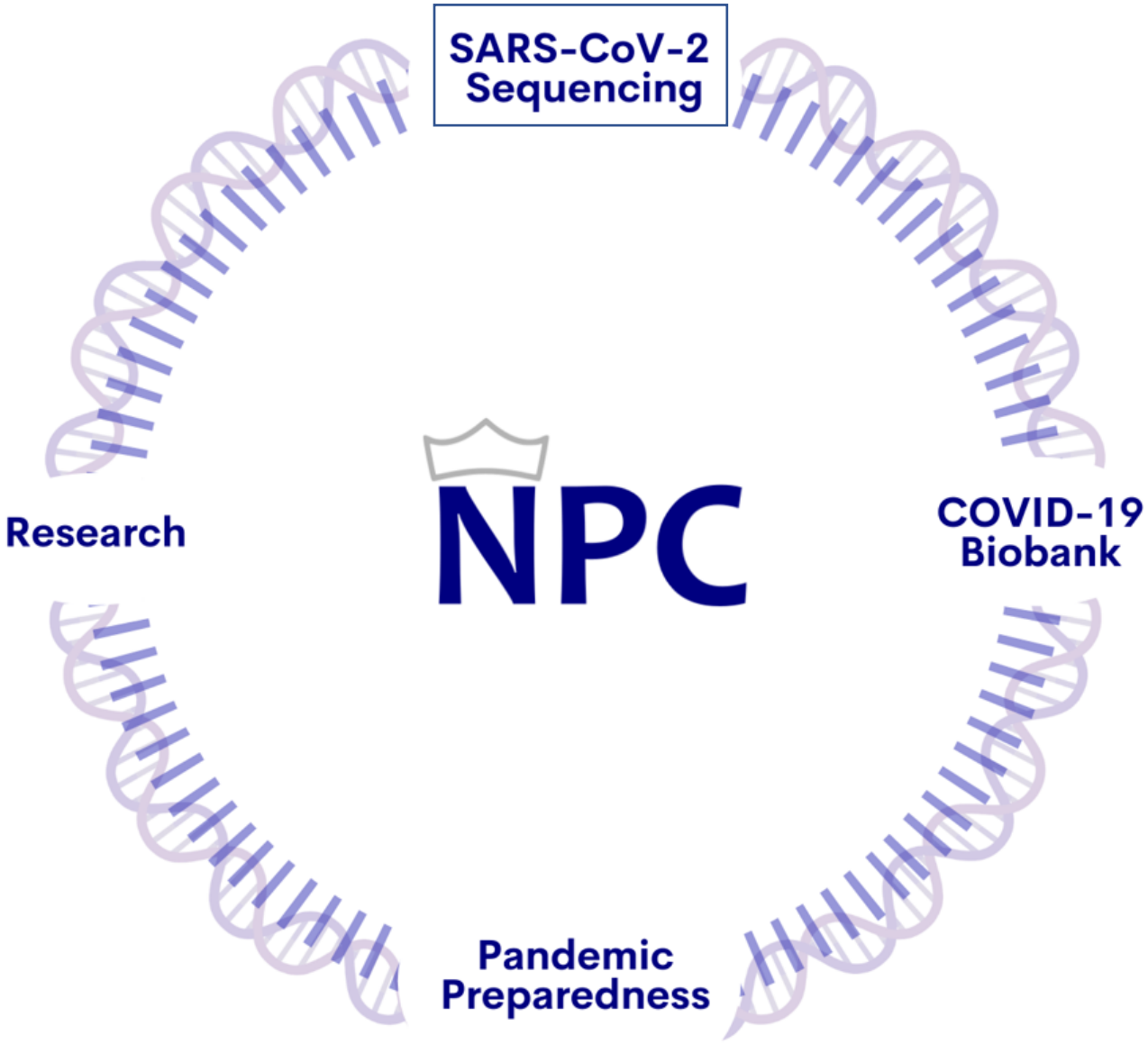
Karolinska Institutet

- Instrument
- Personal
- Kunskap
- Erfarenhet/rutin (Logistik, provhantering, high throughput)
- Stort behov av storskalig WGS av SARS-CoV-2

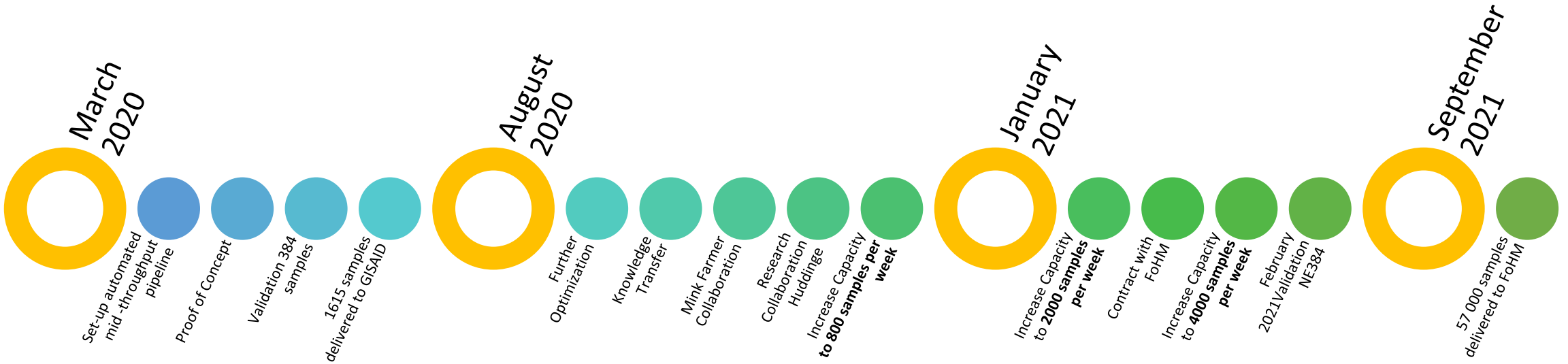
NEW NPC



NPCs verksamhet 2021-



When the magic happens …

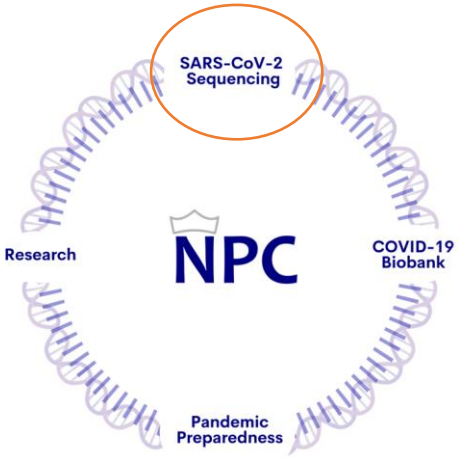


Maike Seifert

SARS-CoV-2 sekvensering

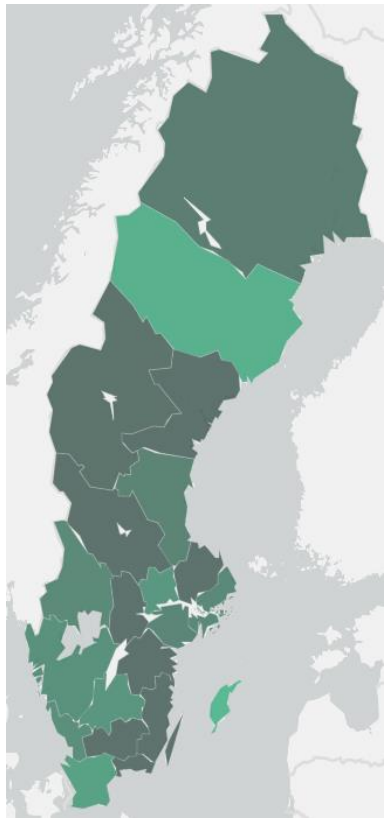
- **Uppdraget**: Nationell övervakning av SARS-CoV-2 – ök med FoHM
- **Proverna:** Under 2021-> hanterat prov från 20 av 21 regioner (12-18 regioner/vecka)
- **Kapacitet/vecka:** 3 200 prov
- **Sekvenserna: >** 125 490 WGS (Feb 2021-Aug 2022)

Central del av det nationella övervakningsprogrammet



SARS-CoV-2 helgenomsekvensering

Uppdrag
2 000 prov/vecka
Ledtid 7-10 dagar



Inkommande prov till NPC för seq

Seq NPC
- Dalarna
- Jönköping
- Värmland
- Kalmar
- Gävleborg
- Norrbotten
- Västernorrland
- Halland
- Kronoberg
- Blekinge
- Västmanland
- Jämtland-Härjedalen
- Örebro

Tidigare seq NPC
- Västerbotten
- Skåne
- Östergötland
- Sörmland
- Stockholm
- Uppsala
- VGR

Inte NPC
- Gotland

Uppdaterade indikationer för helgenomsekvensering av SARS-CoV-2 inom det nationella mikrobiella övervakningsprogrammet (blir gällande 2022-08-01)

Folkhälsomyndigheten

1. Slutenvård
2. Utlandsvistelse
3. Allmän övervakning

   Nivå 1: 10% av positiva
   Nivå 2: Minimum nivå
   Nivå 3: Alla prov (om 7-dagars incidens < 20 fall/100 000 invånare

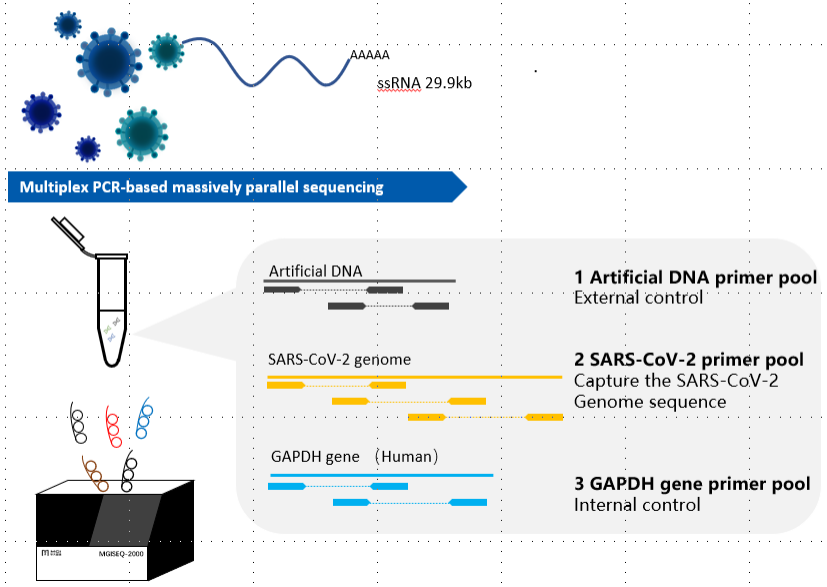
4. Utbrott

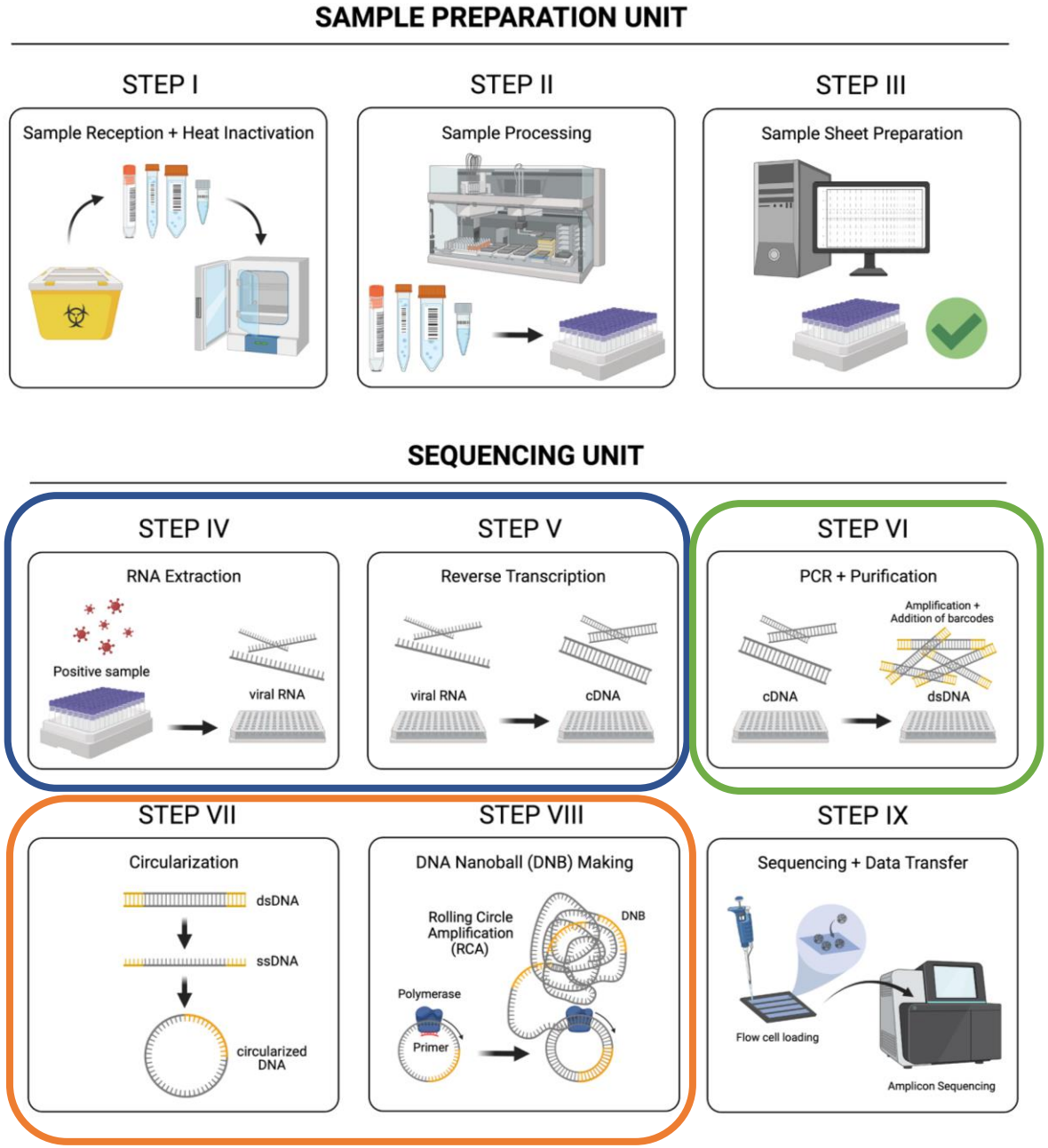
NPC

Karolinska Institutet

Atoplex SARS-CoV-2 Sequencing pipeline: General workflow overview

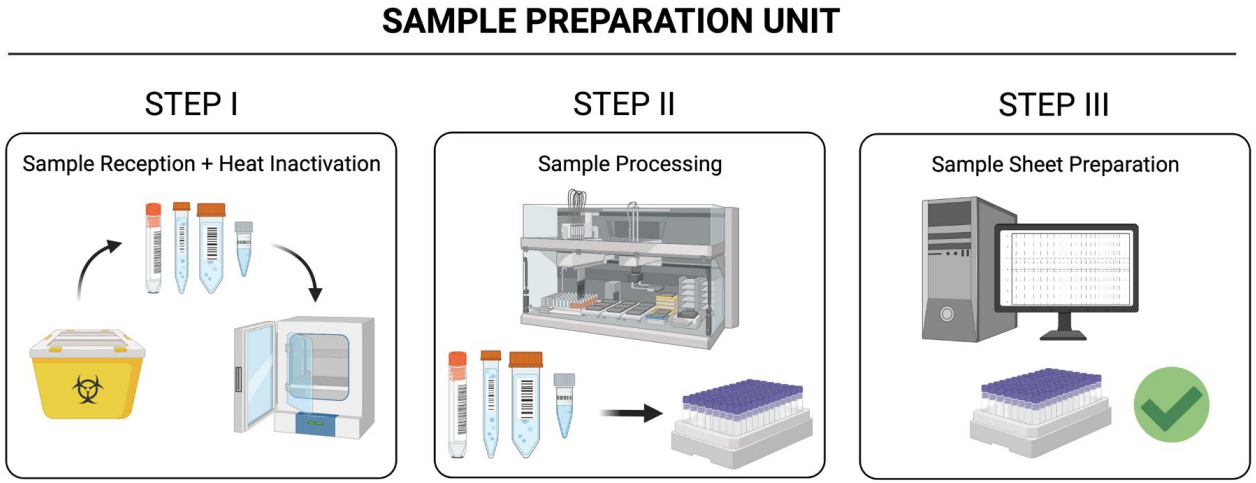
Multiplex PCR Sequencing



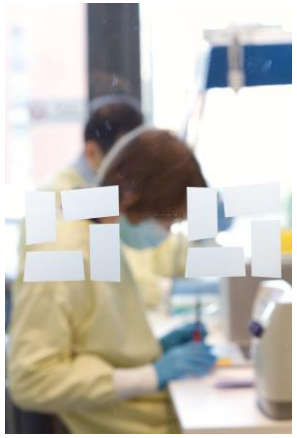


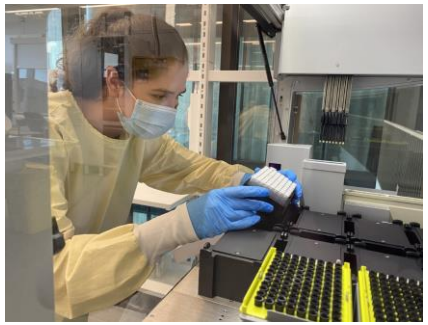
Maike Seifert

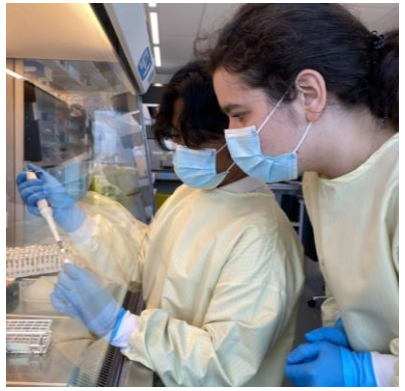
Atoplex SARS-CoV-2 Sequencing pipeline: General workflow overview

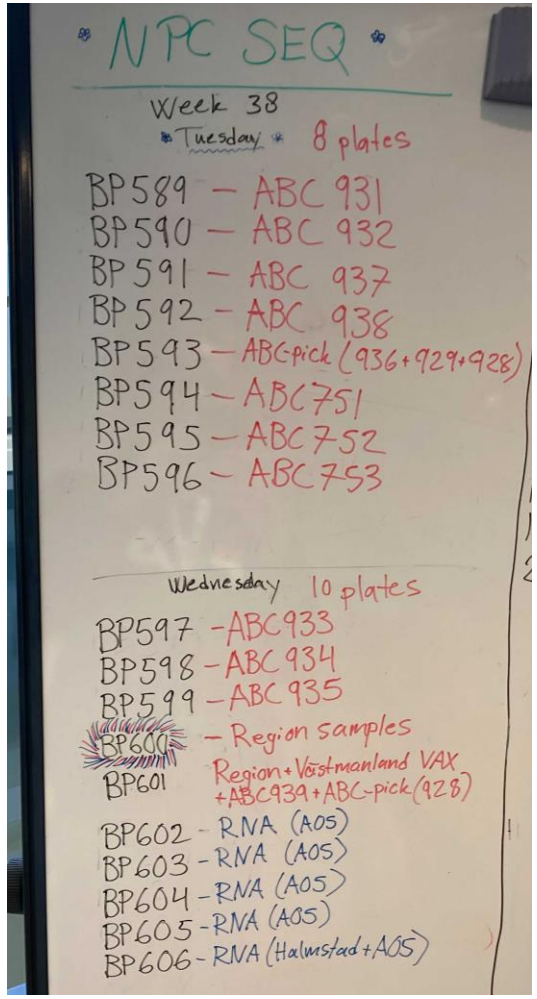


Veckoplanering av kapaciteten för att möta kriterier, behov och avtal

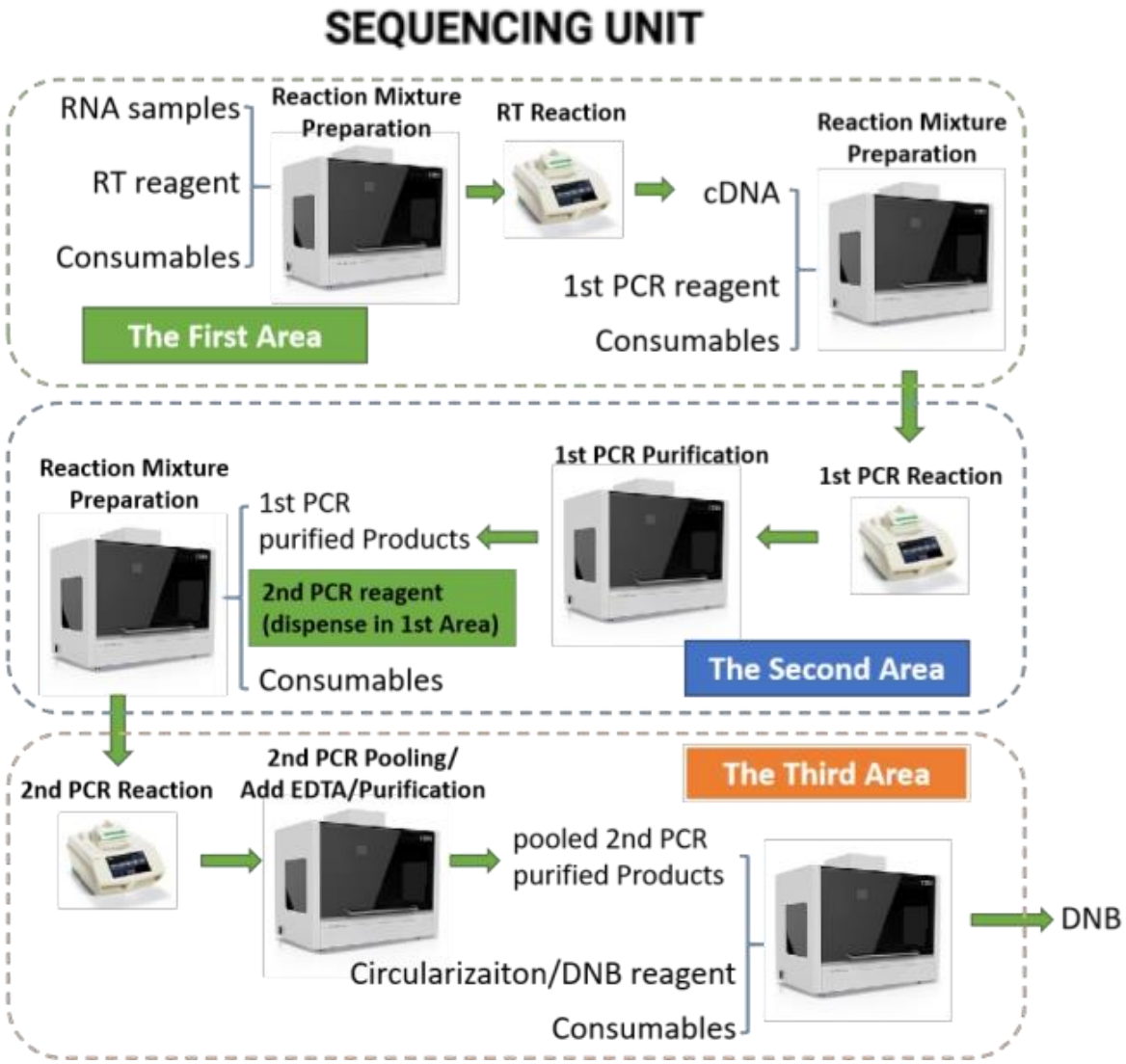


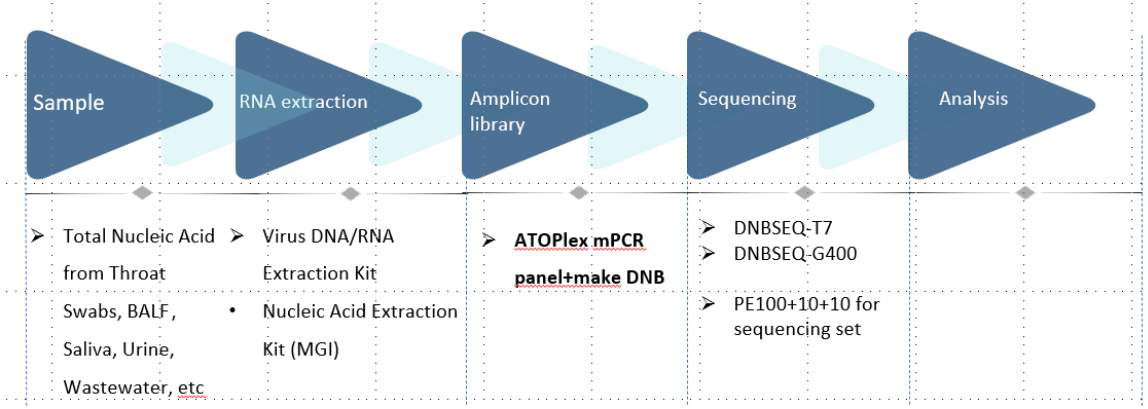






General Workflow overview – Atoplex SARS-CoV-2 Sequencing





Step 1:
- Extraction
- Reverse Transcription
- PCR1
- PCR1 purification

goal: extraction of viral RNA, cDNA synthesis, amplification

Step 2:
- PCR2
- PCR2 purification

goal: attachment of unique barcodes, further amplification

Step 3:
- Circularization
- DNB Making
- Sequencing

goal: generation of DNA Nanoballs via rolling circle amplification, DNBs can be loaded on a flow cell and are ready for sequencing

Maike Seifert

SARS-CoV-2 Sequencing

https://www.gisaid.org/index.php?id=208

Last updated: 28 August 2022 23:04hrs UTC+2

Country / territory	Sequences shared	Reported COVID-19 cases	Reported deaths from COVID-19	% of cases sequenced and shared	Most recent submission, days ago	Most recent collection, days ago	Median days to deposition
United States of America	3,926,250	92,739,935	1,031,832	4.234	1	5	24
United Kingdom	2,801,979	23,493,327	187,761	11.927	1	3	10
Germany	764,966	32,008,122	147,003	2.39	3	6	18
Denmark	558,775	3,268,387	6,888	17.096	4	10	9
France	512,572	33,416,532	150,311	1.534	3	5	26
Canada	416,946	4,135,607	43,505	10.082	3	11	37
Japan	378,844	17,983,797	38,197	2.107	3	9	49
India	233,369	44,389,176	527,556	0.526	2	12	59
Sweden	207,648	2,564,423	19,810	8.097	3	12	29
Brazil	174,146	34,329,600	683,076	0.507	3	17	52
Austria	163,606	4,934,634	20,613	3.315	5	12	22
Spain	157,998	13,323,784	112,246	1.186	1	8	30
Belgium	155,337	4,477,770	32,496	3.469	3	6	18
Switzerland	147,836	4,010,638	13,504	3,686	3	15	16

Sverige 9:e plats i absolut antal sekvenser

Antal prov sekvenserade på NPC: 125 490

Från februari 2021 -> NPC ~60% av SARS-CoV-2 sekvensering i Sverige

2. The Covid Biobank/Förvaring

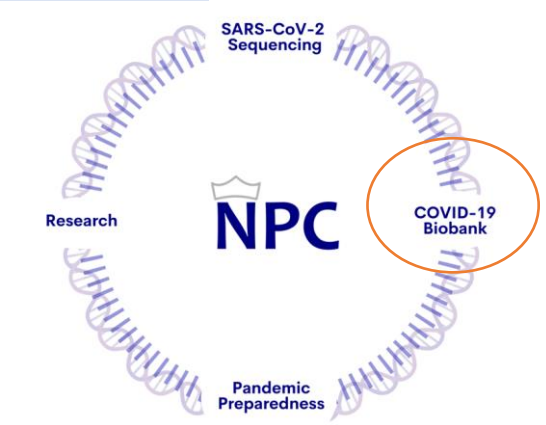
Från 2020

- 621 000 prov; 59 874 positiva
- Stockholm
- VGR
- Västerbotten

Från 2021->

- 20 regioner
- Positiva prov
- ~1 400 000 prov (Aug 2022)

- Regionernas biobanker
- Nära samarbete med ***Biobank Sverige*** och ***Regionerna***
- Förvaringsavtal med NPC/KI



3. Forskning

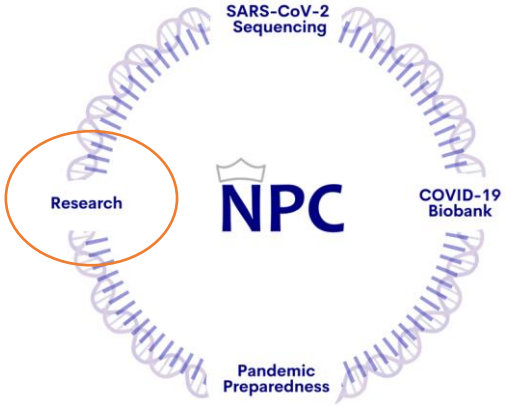
- SÄBO study – Antigentest vs PCR

Completed

- SISCAPA – LC-MS-study with Waters and KTH
- Sequencing samples from Mocambique (NPC, FoHM, PHA in Moc)

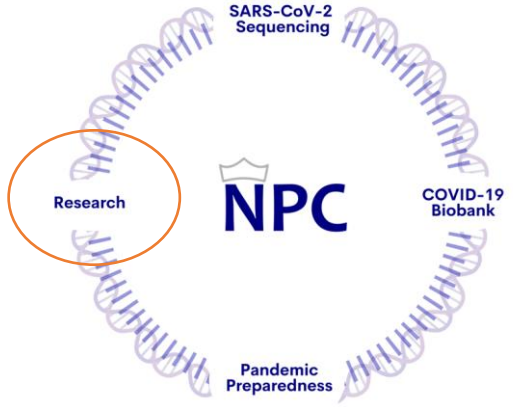
Ongoing

- DESI-SWAB – MS
  - Prototype instrument
  - MS-study with Waters and KTH
  - Covid-19 and beyond
- **Wastewater Immunology**: SARS-CoV-2 surveillance in low-income countries
  - Collaborators: University of New Castle, Wastewater consortium of 15 countries in Latin America, WHO



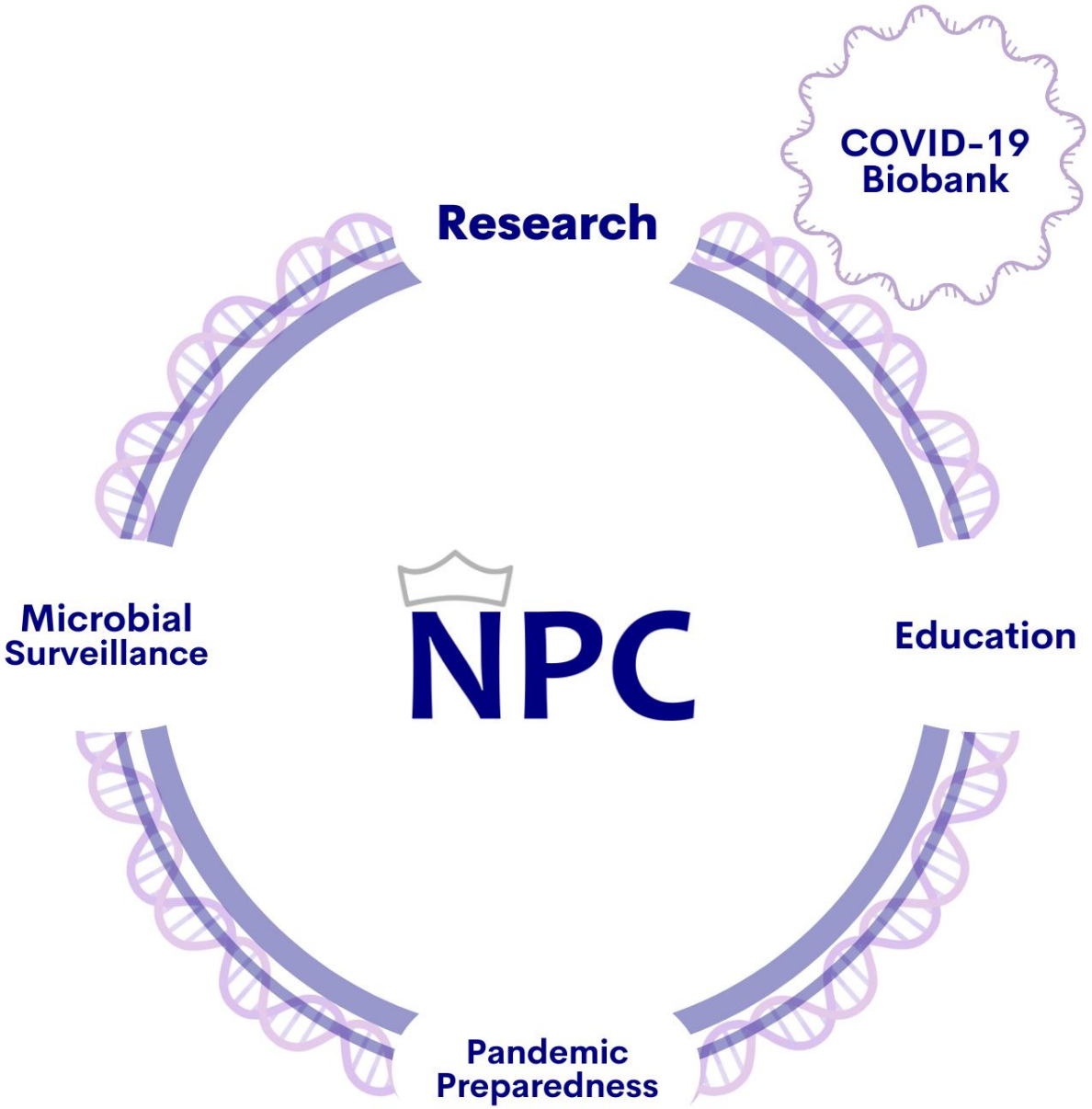
4. Beredskap

- Beredskapsuppdrag för PCR diagnostic – avtal med FoHM
- Del av SciLifeLab Pandemic Laboratory Preparedness (PLP) Network
- Nära samarbete med FoHM och regionerna
- Dagliga verksamheten, instrument, plattformar, personal och erfarenhet från Covid-19 pandemin

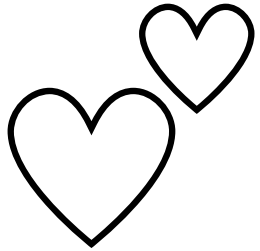


NPCs framtidsvision

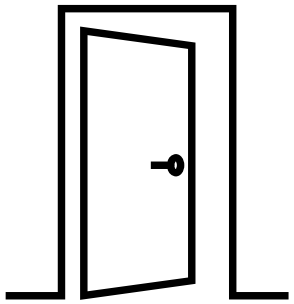
- High-throughput: teknologi, plattformar
- Hög diversitet i kompetens
- Forskning
- Utbildning
- Fortsatt nationellt uppdrag:
  - Samarbete med sjukvårdsregionerna
  - Beredskapsnätverk
  - Internationella samarbeten och nätverk



It always seems impossible until it's done



- Alla som ställde upp och hjälpte till
- Alla öppna dörrar
- Allt som kan åstadkommas och uppnås med engagemang och ett gemensamt syfte och mål



Ett speciellt tack till KI och MTCs administration för all hjälp och support!

Head of Unit:
Marica Hamsten

Project Management and Support

- Study coordination
- Support in study recruitment
- Study logistics
  - Sampling kits out
  - Samples in
- Sample reception
- Lab support
  - Orders & tenders
- Infrastructure
- CTMR biobank

Unit size: 5 persons

Head of Unit:
Fredrik Boulund

IT- and Bioinformatics

- IT-infrastructure and systems development
  - Data collection
  - Data storage
  - Data management
  - Data analysis
- Bioinformatics

Unit size: 10 persons

Head of Unit:
Maike Seifert

Lab Core

- High-throughput sample handling
- Extraction
- Sequencing
- R & D

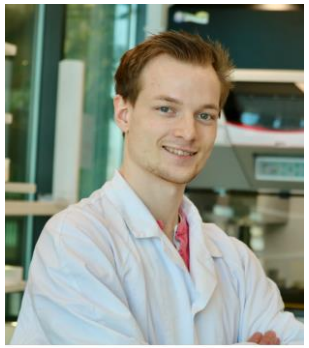
Unit size: 8 persons

CTMR

NPC

NPC

The Crew 2022



Filip Henriksson
Projektsamordnare

Prima Rashid,
Labbkoordinator

Evelina Björninen
Projektsamordnare

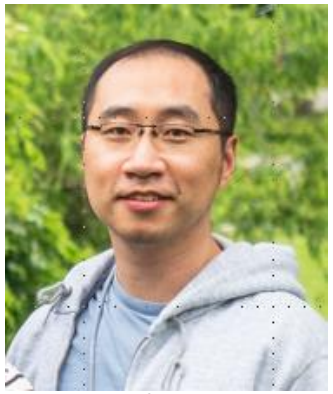
Ali Rihani
Projektledare

Maike Seifert
Leader of the Seq-Pack

Laura Poole
Administrator

Erika Bereczki
Forskningsspecialist

Dorina Ujvari
Forskningsspecialist



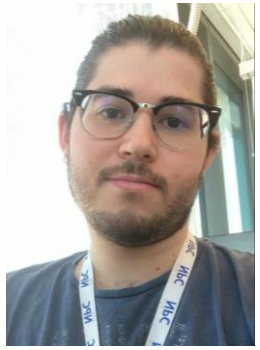
Frank Ning
Postdoc

Yafei Xing
Bioinformatiker

Lottie Jansson Sjöstrand
Forskningsingenjör

Jessica Coleman
The Covid Librerian

Rémi Michelin
Laboratorieingenjör



Javier Herrera
Forskningsassistent

Jacqueline Otto
Forskningsassistent

Defne Yanartas
Forskningsassistent

Alexandra Edwards Henriksson
Forskningsassistent

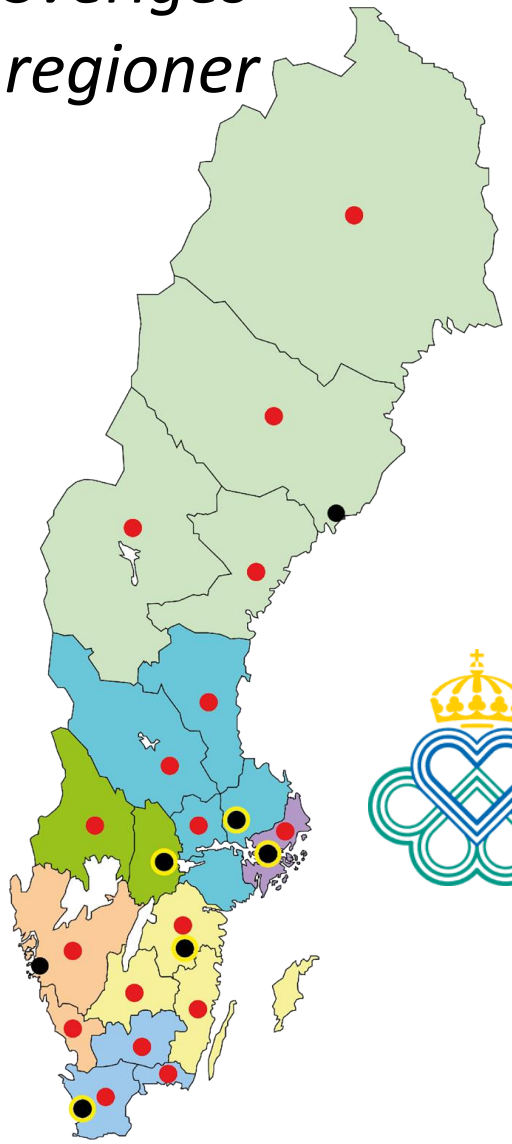
Khue Hua
Forskningsassistent

Johan Lännerström
Forskningsassistent

Joshua Sweidan
Forskningsassistent

Tack!

Sveriges regioner



Karolinska Institutet

Folkhälsomyndigheten

Knut och Alice Wallenbergs Stiftelse

KAROLINSKA UNIVERSITETSLABORATORIET

BIOBANK SVERIGE

MGI

SciLifeLab

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™