Developing COVseq for mass-scale SARS-CoV-2 sequencing

SciLifeLab

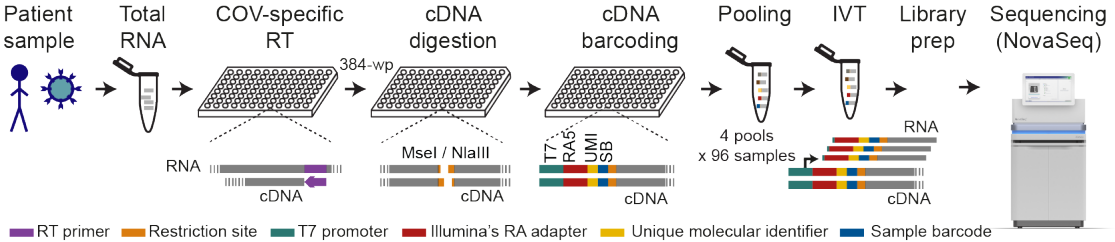
Principal Investigators: Nicola Crosetto (Karolinska Institutet)
Program research area: Viral sequence evolution
Collaborators: Within the program, we anticipate synergy opportunities especially with the Feuk lab in Uppsala as well as with the biobanks

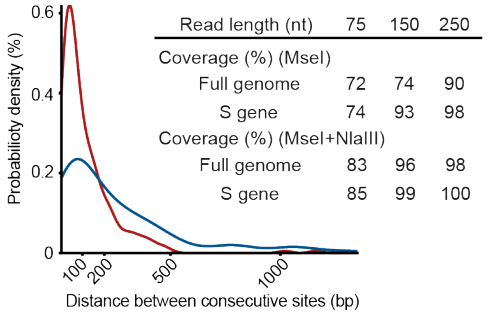
June 5, 2020

- **Aim of project:** To develop a novel method (COVseq) enabling cost-effective large-scale sequencing of SARS-Cov-2 genomes directly from patient samples, building on our recently developed CUTseq approach (*Nat Comm* 2019).

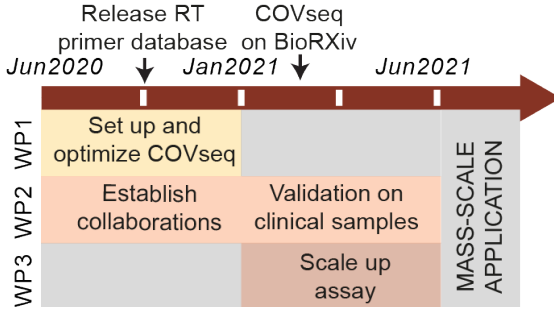
- **Impact on society:** By being able to sequence a large number of SARS-Cov-2 genomes at affordable cost, we can map the evolution of the virus across different populations — especially in higher risk environments such as hospitals and nursing homes — and correlate different genotypes with the type and severity of COVID-19.

- **Approach:** The COVseq workflow includes SARS-Cov-2 specific cDNA synthesis, barcoding, amplification by *in vitro* transcription, and preparation of multiplexed libraries for next-generation sequencing. In this project we aim at (1) developing, (2) validating and (3) scaling up COVseq.





Read length (nt)	75	150	250
Coverage (%) (MseI)			
Full genome	72	74	90
S gene	74	93	98
Coverage (%) (MseI+NlaIII)			
Full genome	83	96	98
S gene	85	99	100



- **Preliminary results:** We are currently implementing the COVseq workflow using cDNA from total human RNA and digesting it with NlaIII. Once this is working, we will spike-in synthetic SARS-CoV-2 RNA and use virus-specific primers to prepare cDNA. We have also received 30 patient samples from one lab in Italy, which we can use for further testing of the method.

A community resource for SARS-CoV-2 structure, interactome and evolution

SciLifeLab

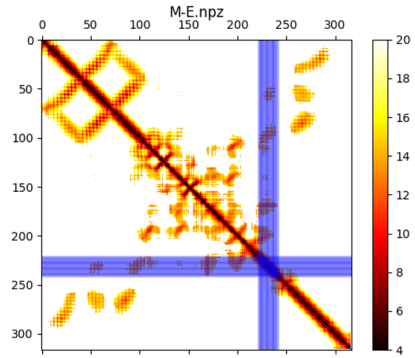
Principal Investigators: Arne Elofsson, Stockholm University
Program research area: Viral Sequence Evolution
Collaborators: Erik Aurell, KTH, Petras Kundrotas, Kansas/SU, Agustin Ure, UNLP/SU Martin Weigt, Sorbonne,

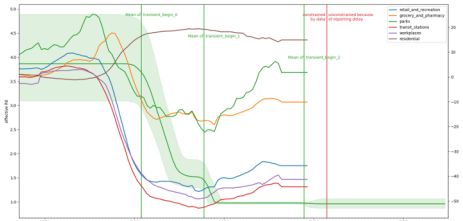
May 2020

Aim of project and impact on society

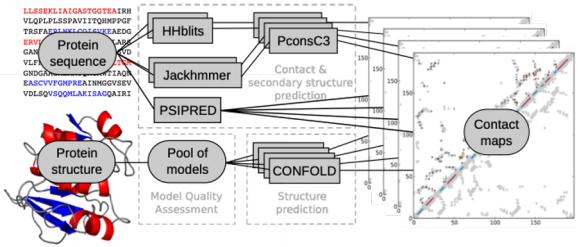
1. Provide a community resource of virus and host sequences related to covid 19.
2. Protein structure (Elofsson). Use soa tools to model covid-19 proteins and work together with the CASP community
3. Protein interactions. Predict virus-virus and virus-host interactions using DCA (Weigt) and ML-methods (Elofsson) and docking (Kundrotas).
4. Studies of epistasis within the viral genome (Aurell+Weigt) using plmDCA
5. *Epidemiological studies - predicting the outcome (Elofsson)*

Results and conclusions.





Short about method if applicable



Papers/manuscripts

Bryant and Elofsson: Estimating the impact of mobility patterns on COVID-19 infection rates in 11 European countries, submitted
Zeng et al. "Global analysis of more than ...." manuscript.

Resource pages:

https://covid19.bioinfo.se
https://gitlab.com/ElofssonLab/sars-cov-2

Rapid cDNA and direct RNA sequencing of SARS-CoV-2 using Oxford Nanopore

Principal Investigators: Lars Feuk, Uppsala University
Program research area: Viral Sequence Evolution
Collaborators: **Anna Petri (NGI UU), Adam Ameur (NGI UU), Olga Vinnere Pettersson (NGI UU), Ellen Sherwood (NGI STO), Carl Rubin (NGI STO)**

May 2020

Aim of project and impact on society (Goals/Objectives, project plan)

- Aim 1: Set up the ARTIC protocol for rapid SARS-CoV-2 cDNA sequencing at NGI to:
  - Enable small-scale research projects
  - Establish a protocol that can be easily transfered to other labs
  - Report sequences to international databases (Nextstrain, GISAID)
- Aim 2: Set up the capacity to perform direct RNA sequencing of SARS-CoV-2
  - Base modification detection

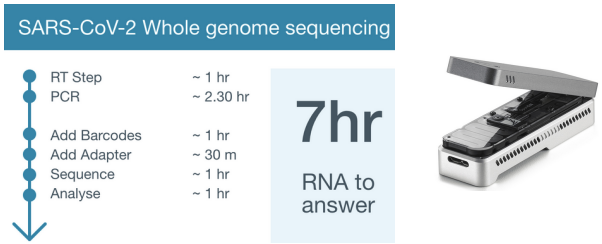
Results and conclusions if any already (preferably as figures, charts, tables….)

- All reagents have been ordered and most are in place
- Expect first test of protocol end of next week (depending on on-going lab move)
- Initial test will be run on purchased control RNA

Short about method if applicable (preferably as a figure)

cDNA

- Multiplex PCR, 400bp amplicons
- Read-out on Oxford Nanopore MinION
- 24 bar-coded samples/flow cell



Direct RNA

Oxford nanopore direct RNA sequencing

Analysis of essential genes and validity as drug targets

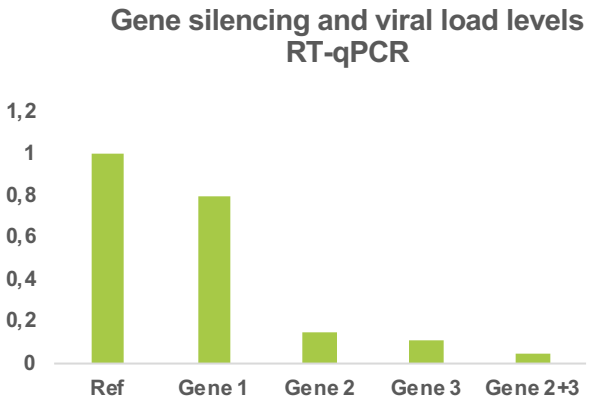
Principal Investigators: Tomas Nyman (Karolinska University), Theodor Pramer (Biomedrex)
Program research area: Viral Sequence Evolution, analysis of essential genes and validity as drug targets
Collaborators: SciLifeLab Protein Science Facility, Tufts University, Biomedrex

May 2020

Aim of project and impact on society

- Verify optimal drug targets
- Evaluate drug target synergies
- Minimize drug resistance from these
- Aid drug research focus
- Method for future outbreaks

Method has been used for, HIV-1, HBV, HSV-1/2 and now adopted to RNA-virus



First set of 15 guide RNA targeting different genes