Application of SARS-CoV-2 NGS

ErasmusMC



8 May 2020

Bas Oude Munnink, David Nieuwenhuijse, Reina Sikkema, Marion Koopmans



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101003589.

Aims



- Reconstruct the trajectory of SARS-CoV-2 emergence and spread nationally and internationally
- Application of NGS in public health policy making and infection prevention policies
- Monitoring relevant mutations: genotype to phenotype
- Need to do this fast and to share data near to instantly



Amplicon based sequencing

PROTOCOL

Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples

Joshua Quick¹, Nathan D Grubaugh², Steven T Pullan³, Ingra M Claro⁴, Andrew D Smith¹, Karthik Gangavarapu², Glenn Oliveira⁵, Refugio Robles-Sikisaka², Thomas F Rogers^{2,6}, Nathan A Beutler², Dennis R Burton², Lia Laura Lewis-Ximenez⁷, Jaqueline Goes de Jesus⁸, Marta Giovanetti^{8,9}, Sarah C Hill¹⁰, Allison Black^{11,12}, Trevor Bedford¹¹, Miles W Carroll^{3,13}, Marcio Nunes¹⁴, Luiz Carlos Alcantara Jr.⁸, Ester C Sabino⁴, Sally A Baylis¹⁵, Nuno R Faria¹⁰, Matthew Loose¹⁶, Jared T Simpson¹⁷, Oliver G Pybus¹⁰, Kristian G Andersen^{2,5} & Nicholas J Loman¹

¹Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, UK. ²The Scripps Research Institute, La Jolla, California, USA. ³Public Health England, National Infection Service, Porton Down, Salisbury, UK. ⁴Department of Infections Disease and Institute of Tropical Medicine, University of Saö Paulo, Saö Paulo, Brazil. ⁵Scripps Translational Science Institute, La Jolla, California, USA. ⁴Massachusetts General Hospital, Boston, Massachusetts, USA. ⁷Instituto Dowaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. ⁹Fundação Oswaldo Cruz (FIOCRUZ), Salvador, Brazil. ⁹University of Mome, Tor Vergata, Italy. ¹⁰Department of Zoology, University of Oxford, Oxford, UK. ¹¹Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. ¹²Department of Epidemiology, University of Washington, Seattle, Washington, USA. ¹³University of Southampton, South General Hospital, Southampton, UK. ¹⁴Instituto Evandro Chagas, Belem, Brazil. ¹⁵Paul-Ehrlich-Institut, Langen, Germany. ¹⁶DeepSeq, School of Life Sciences, University of Nottingham, UK. ¹⁷OICR, Toronto, Canada. Correspondence should be addressed to N.J.L. (n.]. Joman@bham.ac.uk).



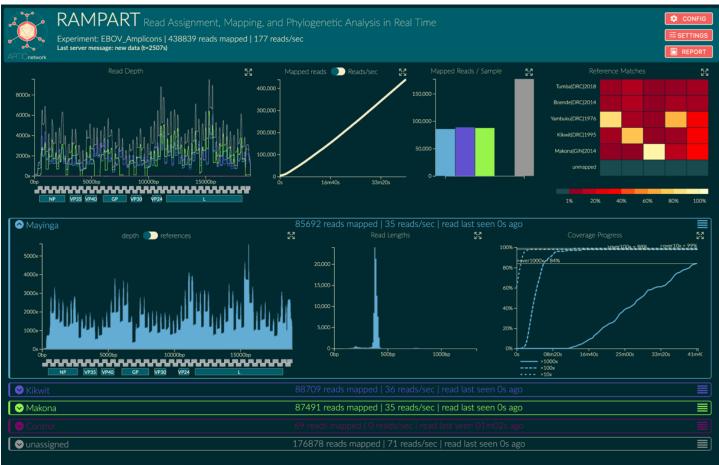


83 overlappende amplicons500bp75 bp overlap





Data analysis



AMPART is built by James Hadfield, Nick Loman and Andrew Rambaut as part of the ARTIC Network project Funded through The Wellcome Trust Collaborators Award 206298_A_17_Z. RECOVER PORT

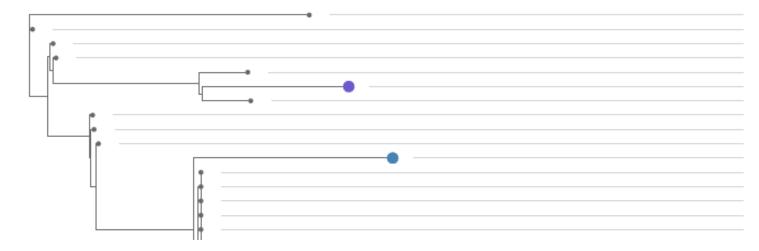
Custom script to automatically collect data from the machine and transfer to the server

Real-time automated dataanalysis:

- Demultiplex
- Trim primers
- Align to reference
- Generate consensus sequence (10x, 30 and 100x coverage)



Phase 1: Initial testing of travelers according to the WHO and ECDC case definitions



BetaCoV_Finland_FIN-25_2020_EPI_ISL_412971_2020-02-25 BetaCoV_HongKong_VM20002345_2020_EPI_ISL_409027_2020-02-03 BetaCoV_Italy_CDG1_2020_EPI_ISL_412973_2020-02-20 BetaCoV_Brazil_SPBR-01_2020_EPI_ISL_412964_2020-02-25 BetaCoV_Mexico_InDRE_01_2020_EPI_ISL_412972_2020-02-27 BetaCoV_Netherlands_Diemen_2020 BetaCoV_Germany_Baden-Wuerttemberg-1_2020_EPI_ISL_412912_2020-02-25 BetaCoV_Kanagawa_1_2020_EPI_ISL_402126_2020-01-14

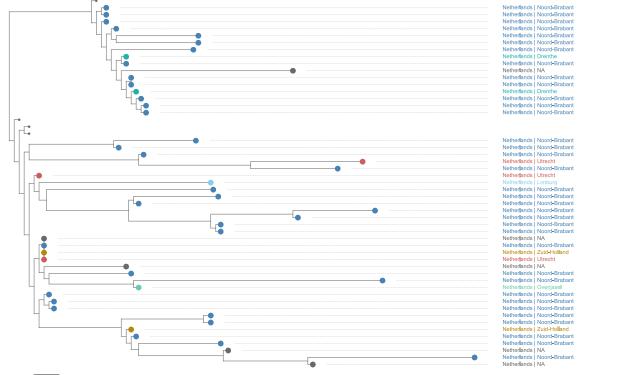
BetaCoV_Kanagawa_1_2020_EPI_ISL_402126_2020-01-14 BetaCoV_HongKong_VM20001218_2020_EPI_ISL_408995_2020-01-24 BetaCoV_Munich_BavPat1_2020_EPI_ISL_406862_2020-01-28

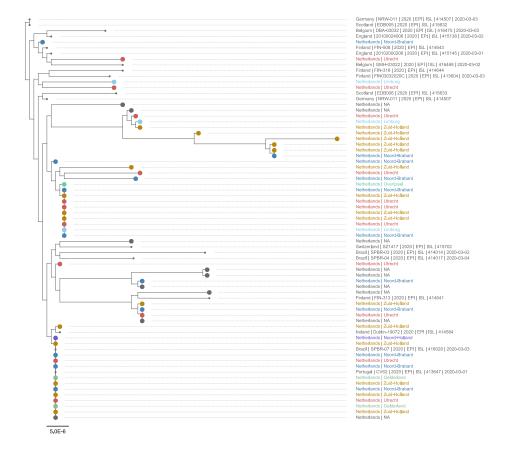
BetaCoV_Netherlands_Tilburg_2020

BetaCoV_HongKong_VM20001794_2020_EPI_ISL_409001_2020-01-29 BetaCoV_HongKong_VM20001403_2020_EPI_ISL_409000_2020-01-26 BetaCoV_HongKong_VB20026565_2020_EPI_ISL_409024_2020-01-31 BetaCoV_HongKong_VM20001988_2020_EPI_ISL_409020_2020-01-30 BetaCoV_HongKong_VM20002162_2020_EPI_ISL_409023_2020-01-31



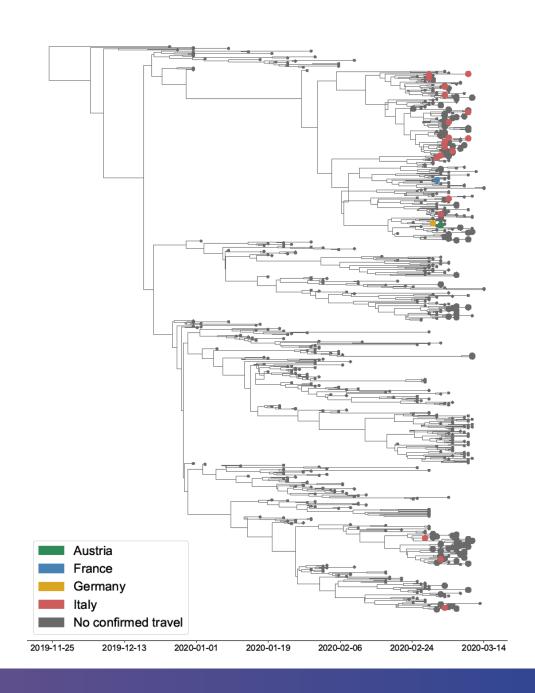
Phase 2: Inclusion of patients hospitalized with severe respiratory infections



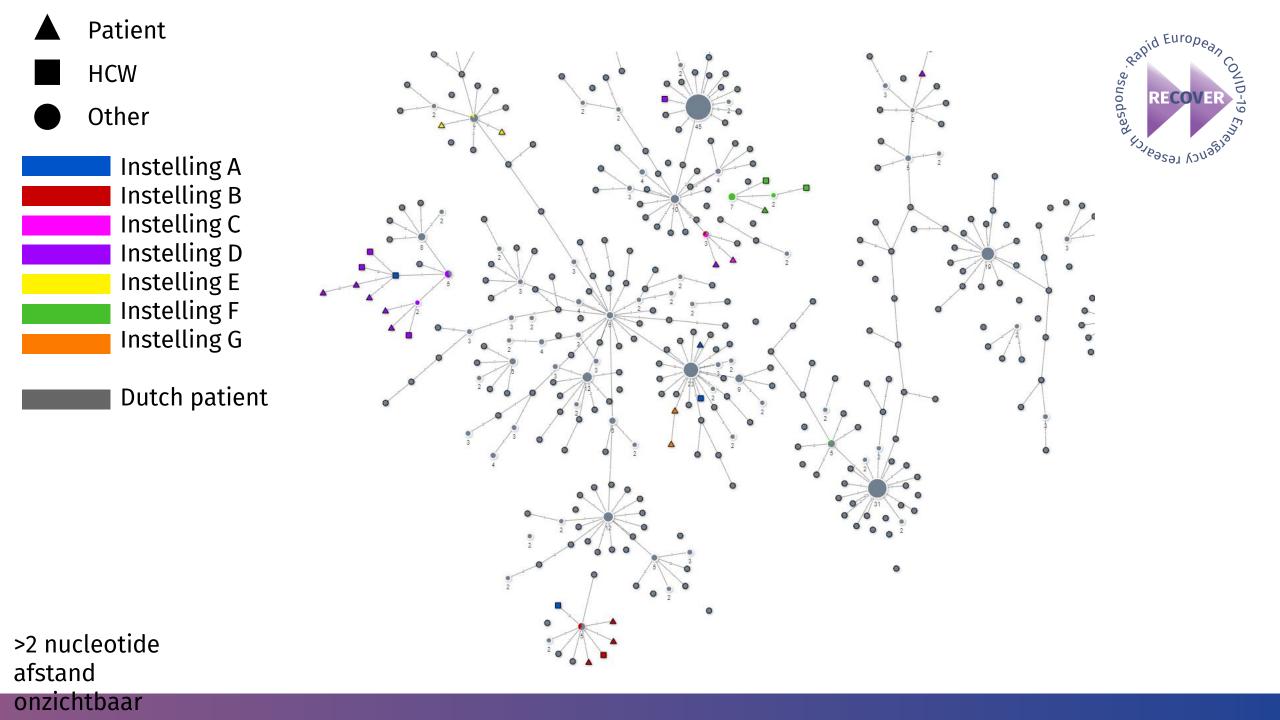


5.0E-6

Phase 3: Systematic sequencing during exponential growth phase





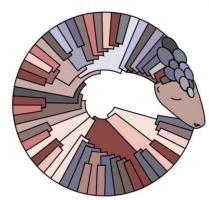




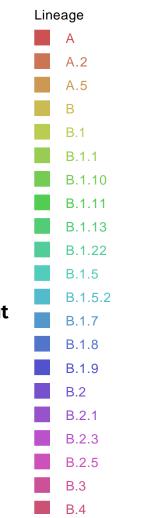
Dutch situation report

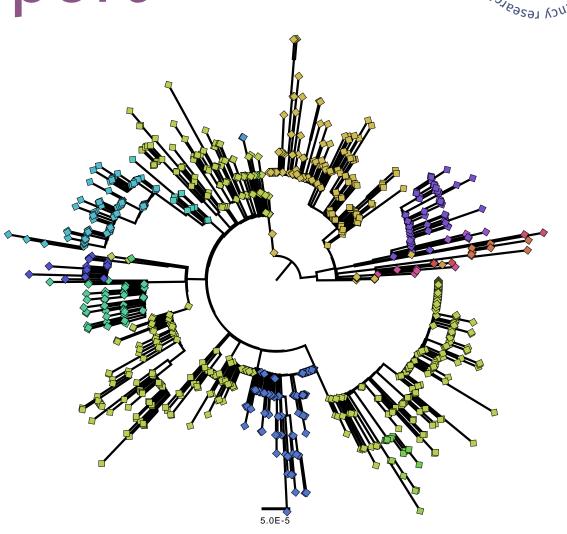
pangolin

Phylogenetic Assignment of Named Global Outbreak LINeages



Aine O'Toole and Andrew Rambaut







Ongoing..

- Monitoring of relevant mutations
- Longitudinal monitoring patients
- Househld studies
- Animal studies

Ongoing..

- Monitoring of relevant mutations
- Longitudinal monitoring patients
- Househld studies
- Animal studies

Consortium partners









