SEQAFRICA Virtual Training Course

Zoom and Slack Online Platforms

17-28 May 2021

Course information

Title: SARS-CoV-2 whole genome sequencing

Language of instruction: English.
Offered as: Webinar with lectures and exercises.
Duration of course: 4 x ½ days.
Responsible: Rene S. Hendriksen (DTU), rshe@food.dtu.dk
Course co-responsible: Pernille Nilsson (DTU), Anthony Smith (NICD, South Africa), Jinal Bhiman (NICD, South Africa), Marco van Zwetselaar (KCRI, Tanzania), Beverly Eygir (NMIMR, Ghana), Iruka N. Okeke (UI, Nigeria).

General course objectives:

The course introduces and cover all aspects of the entire WGS workflow for SARS-CoV-2 starting with a respiratory sample and finishing with completely analysed DNA sequence/genome.

The participant will upon completion of the course know all the steps included in preparing and conducting WGS using Illumina short read technology, which will include the wet laboratory procedures, computational genome assembly and the use of online tools to assess phylogenies and mutations in the global context.

Learning objectives:

A participant who has met the objectives of the course will be able to:

- Describe wet laboratory and computational workflow to generate and assemble SARS-CoV-2 genomes
- Perform wet laboratory protocols
- Perform drag-and-drop bioinformatics using online analysis tools and interpret the results

Content:

The course covers a detailed run through the sequencing workflow, starting with a respiratory sample to completed analysis of raw reads or assembled genome and will give the participants a thorough understanding of the process for short read technologies.

The course will comprise of lectures and hands-on exercises that the participants will have to complete and submit answers to in between course days.

Course literature:

No literature required.

Audience:

Users with some experience/knowledge of WGS and WGS data (e.g. attended SEQAFRICAs Introduction to WGS in AMR surveillance).

Day 1: Monday – 17 May 2021 – Sequencing workflow overview and wet lab methods Join Zoom call and Slack channel to attend		
Time (CET)	**Content**	**Lecturer/ Facilitator**
08.45 – 09.00	**Joining the call** – Assistance will be provided at this time to help participants join	
09.00 – 09.15	**Welcome and Introduction (Live)**	Pernille Nilsson (DTU, Denmark)
09.15 – 09.45	[1] **SARS-CoV-2 genomic surveillance:** From respiratory sample to SARS-CoV-2 genome. (Pre-recorded)	Jinal Bhiman (NICD, South Africa)
09.45 – 10.00	**BREAK**	
10.00 – 10.30	[2] **RNA extraction:** From respiratory sample to RNA. (Pre-recorded)	Noxolo Ntuli (NICD, South Africa)
10.30 – 11.00	[3] **cDNA synthesis and tiling PCR:** Method and background for cDNA synthesis using random hexamers followed by tiling PCR using primer pools with >170 individual primers (Pre-recorded)	Boitshoko Mahlangu (NICD, South Africa)
11.00 – 11.15	[4] **Quantification and Amplicon Generation** (Pre-recorded)	Bright Adu, Frank Oteng/Noguchi team (NMIMR, Ghana)
11.15 – 11.45	**BREAK**	
11.45 – 12.15	[5a] **Library preparation:** Going from high quality DNA to sequencing libraries. "Hands-on" and theory. Description of library preparation using the Nextera Flex kit (Pre-recorded)	Bright Adu, Frank Oteng/Noguchi team (NMIMR, Ghana)
12.15 – 12.45	[5b] **Library preparation:** Going from high quality DNA to sequencing libraries. "Hands-on" and theory. Description of library preparation using the COVIDSeq and the Thermo Fisher kits (Pre-recorded)	Thabo Mohale, Zama Khumalo (NICD, South Africa)
12.45 – 13.00	**Q&A and Wrap-up (Live)**	Pernille Nilsson (DTU, Denmark)

Day 2: Wednesday – 19 May 2021 – Illumina sequencing and accessing data		
Join Zoom call and Slack channel to attend		
09.45 – 10.00	**Joining the call** – Assistance will be provided at this time to help participants join	
10.00 – 10.15	**Welcome and Introduction (Live)**	Pernille Nilsson (DTU, Denmark)
10.15 – 10.35	[6a] **Illumina sequencing:** Hands-on how to load the NextSeq machine with your prepared libraries (Pre-recorded video).	Arshad Ismail, Zama Khumalo (NICD, South Africa)
10.35 – 10.55	[6b] **Illumina sequencing:** Hands-on how to load the MiSeq machine with your prepared libraries (Pre-recorded video).	Bright Adu, Frank Oteng/Noguchi team (NMIMR, Ghana)
10.55 – 11.30	**BREAK**	
11.30 – 12.00	[7] **Downloading data:** Once the sequencing run is finished, how do you get your data? (Pre-recorded video / Demonstration)	Stanford Kwenda (NICD, South Africa) Bright Adu, Frank Oteng/Noguchi team (NMIMR, Ghana)
12.00 – 12.15	[7Q] **Quiz:** Introduction to quiz covering the wet lab steps. QC of samples through in-house and COVIDSeq methods following and/or prior to library prep. ***You are required to hand in response via Survey Monkey prior to day 4.***	Arshad Ismail, Zama Khumalo, Thabo Mohale (NICD, South Africa) Bright Adu, Frank Oteng and Noguchi team (NMIMR, Ghana)
12.15 – 12.30	**Q&A and Wrap-up (Live)**	Pernille Nilsson (DTU, Denmark)

Day 3: Friday – 21 May 2021 – SARS-CoV-2 WGS bioinformatics workflows and online tools		
Join Zoom call and Slack channel to attend		
08.45 – 09.00	**Joining the call** – Assistance will be provided at this time to help participants join	
09.00 – 09.15	**Welcome and Introduction (Live)**	Pernille Nilsson (DTU, Denmark)
09.15 – 09.45	[8] **Recap on the vet lab workflow.** (Live)	Jinal Bhiman (NICD, South Africa)
09.45 – 10.15	[9] **Galaxy pipeline**: Bioinformatics pipeline used for SARS-CoV-2 genome assemble using a reference. QC of sequence outputs from Galaxy (Live)	Cathrine Scheepers, (NICD, South Africa)
10.15 – 10.30	**BREAK**	
10.30 - 11.00	[10] **Exatype pipeline**: Bioinformatic pipelines used for SARS-CoV-2 genome assemble using a reference. QC of sequence outputs from Exatype (Pre-recorded)	Simon Travers (Exatype)
11.00 – 11.30	[11a] **Introduction to Nextstrain online tool:** Demonstration of data analysis at Nextstrain. (Pre-recorded)	Bright Adu, Frank Oteng/Noguchi team (NMIMR, Ghana)
11.30 – 12.00	**BREAK**	
12.00 – 12.30	[11b] **Introduction to PANGOLin and COVDB online tools:** Demonstration of data analysis at PANGOLin and Stanford University – implication of mutations reported in literature (vaccine escape etc.) (Pre-recorded)	Daniel Amoako (NICD, South Africa)
12.30 – 12.45	[9_10E] **Introduction to Exercise:** Going from raw reads to analyzed genome assemblies Assemble WGS, generate FASTA, look at QC, assign lineage and clade, compare Galaxy and Exatype outputs. Low, medium and high quality test set for generating the QC report will be provided. ***You are required to hand in results via Survey Monkey prior to day 4.***	
12.45 -13.00	**Q&A and Wrap-up (Live)**	Pernille Nilsson (DTU, Denmark)

	Day 4: Friday– 28 May 2021 – Mutant impact assessment, data sharing and review of exercises/quizzes Join Zoom call and Slack channel to attend	
08.45 – 09.00	**Joining the call** – Assistance will be provided at this time to help participants join	
09.00 – 09.15	**Welcome and Introduction (Live)**	Pernille Nilsson (DTU, Denmark)
09.15 – 09.45	[12] **Introduction to PyMoL:** Predictive assessment of impact of mutations based on spike structure (For novel mutations). (Pre-recorded)	Constantinos Kurt Wibmer (NICD, South Africa)
09.45 – 10.00	[12E] **Exercise:** Plot given set of mutations on structure and suggest functional role for these. (Live)	Constantinos Kurt Wibmer (NICD, South Africa)
10.00 – 10.45	**BREAK**	
10.45 - 11.15	[13] **Data sharing and uploading to GISAID:** Overview of the GISAID database, polices and uploading (including compulsory metadata). (Pre-recorded)	Anne von Gottberg (NICD, South Africa)
11.15 – 11.30	**Going through results from Quiz 7Q** (Live)	
11.30 – 12.00	**BREAK**	
12.00 – 13.00	**Going through results from 11E** (Live)	
13.00 – 13.15	**Q&A and Close (Live)**	Pernille Nilsson (DTU, Denmark)