

Summary report on the

Subregional training on SARS-CoV-2 sequencing and molecular phylogenetics for national influenza centres in the Eastern Mediterranean Region

WHO-EM/CSR/516/E

Abu Dhabi, United Arab Emirates
5–9 December and 12–16 December 2021



REGIONAL OFFICE FOR THE

World Health
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1. Introduction

All countries in the WHO Eastern Mediterranean Region are affected by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that was first encountered in December 2019 and continues to pose multiple health challenges across the globe, including in the Eastern Mediterranean Region, with a progressive increase in cases and deaths around the world. SARS-CoV-2 infection spreads rapidly and acquires new mutations, increasing human-to-human transmissions. Therefore, laboratory preparedness regarding a sequencing platform is indispensable for monitoring the current coronavirus disease (COVID-19) pandemic. The WHO Regional Office for the Eastern Mediterranean supports and coordinates closely with the 22 countries and territories in the Region to reduce the circulation and transmission of SARS-CoV-2 nationally and regionally by promoting research and innovation, strengthening SARS-CoV-2 surveillance and monitoring, and utilizing data to inform SARS-CoV-2 prevention and control policies and programmes to protect the vulnerable.

Viruses are constantly changing, and this includes SARS-CoV-2. These variations in genetic material occur over time and lead to the emergence of new variants that may have different features. SARS-CoV-2 genome sequencing is critical for the identification and monitoring of new variants in the Region and can help to guide the public health response to the pandemic and improve strategies to reduce the burden of COVID-19. In addition, genomic sequencing can be used to investigate outbreak dynamics, including changes in the size of the epidemic over time, spatiotemporal spread and transmission routes. Genomic sequencing can also help in the design of diagnostic assays, drugs and vaccines.

Increased understanding of the potential of genomic sequencing to improve public health is leading countries to establish genome sequencing facilities. Currently, 14 out of the 22 countries/territories in

the Region have genome sequencing capacities to detect and monitor new variants, while WHO has been facilitating the shipment of samples from eight countries with no genome sequencing capacity to two regional reference laboratories in Oman and the United Arab Emirates, in addition to other internal reference laboratories.

As requested by its Member States, WHO is scaling up technical support to enhance sequencing capacity through training, mentoring and the provision of supplies. Accordingly, the WHO Regional Office held a 5-day hands-on training programme on the implementation of next generation sequencing (NGS) in eight countries (Afghanistan, Iraq, Lebanon, Libya, Somalia, Sudan, the Syrian Arab Republic and Yemen) before its implementation in the national influenza centres of these countries. The training was held at the regional reference laboratory at Sheikh Khalifa Medical City in Abu Dhabi, United Arab Emirates, on 5–9 December and 12–16 December 2021.

The objective of the training was to train laboratory technicians in NGS from library preparation to sequence analysis, including;

- how to set up and install the MinION Mk1C system for NGS and provide practical training on the MinION Mk1C system using a SARS-CoV-2 control sample sequence;
- how to handle the data collected from the MinION Mk1C system and perform sequence analysis;
- how to collect metadata for sequencing samples and which metadata are essential to enabling the best use of SARS-CoV-2 for the pandemic response; and
- how to share sequencing data through international sequence-sharing platforms.

2. Process and methods

Training design

The training materials, standard operating procedures (SOPs) and methods were designed based on discussions between the organizers from the WHO Regional Office for the Eastern Mediterranean and Sheikh Khalifa Medical City using teleconferencing, a shared drive and emails. The discussions lasted several weeks and aimed to:

- prepare the SOPs for the training;
- prepare the materials and presentations for the training, including all aspects of a typical NGS workflow from sample preparation to NGS library preparation, sequencing and data analysis;
- agree on the training's objectives and duration; and
- review and adapt a SARS-CoV-2 sequencing training programme to fit with the platform that will be used by participants in their countries.

Training methodology

The training was divided into six sections.

1. An overview of molecular biology and the SARS-CoV-2 genome. This session covered the basic molecular virology of SARS-CoV-2, including genetics, genome organization, expression and replication, and evolutionary rate. The section also reviewed how SARS-CoV-2 genome sequencing has been used at different stages of the COVID-19 pandemic and its potential future applications.
2. Sequencing platforms. NGS platforms are more appropriate for whole genome sequencing. Sequencing platforms that have been commonly used to date for SARS-CoV-2 include those from Illumina, Ion Torrent and Oxford Nanopore Technologies. The training focused on Oxford Nanopore Technologies, which will be

used in the countries, and included an overview of the MinION Mk1C system, the theoretical aspects and mechanism of the technology and troubleshooting MinION sequencing.

3. Laboratory work. This section aimed to train the participants on NGS, and included sample preparation, multiplex PCR, library preparation, rapid barcoding, and MinION setup and sequencing.
4. SARS-CoV-2 variants. This section covered the main steps for sequencing data and determining the type of variant across all virus genomes sequenced to date and the thousands of mutations that have emerged since the start of the pandemic, which in turn have given rise to thousands of different variants. Several variants have been identified that appear to increase transmissibility and potentially have an impact on disease severity. The section also covered the impact of variants on diagnostics and vaccines.
5. Data analysis and phylogeny. This section covered how to handle sequencing data from NGS, which generates large volumes of data, and genome assembly from raw data using a software pipeline. The participants were able to construct phylogenetic trees using FigTree and MEGA software. They were also trained to be able to use the nomenclature systems for SARS-CoV-2 clades/lineages using Nextstrain and Pangolin, which provide an interactive display of the evolution and geographical diversity of SARS-CoV-2 through the naming of different phylogenetic clades.
6. Reporting sequencing data to public genome repositories. The rapid sharing of SARS-CoV-2 genome sequence data, together with metadata, will maximise the impact of genomic sequencing in the public health response. Shared metadata should always include at least the date and location of sample collection, but additional metadata will greatly increase the potential applications of the sequence data.

Evaluation of the training

Efforts were made to make the training interesting and interactive for the participants. Evaluation was conducted systematically throughout the training and included: end-of-day immediate participant feedback comments on what went well and what could be improved; instructor observations; and a daily instructors' meeting to review the participants' comments and plan for the next day.

3. Results

Materials developed

Modified training materials and SOPs were developed, which can be used as a basis for further training on Oxford Nanopore Technologies or other NGS platforms. The training materials were shared with the participants.

Outcomes

By the end of the training, participants were: able to understand the basic concepts of NGS; familiar with how to set up and install the MinION Mk1C system and handle the data collected from the system; and able to share sequencing data via sequence-sharing platforms.

Addressing training needs

The training sessions, discussions and participant feedback were used to address the main gaps in using NGS. Training recommendations were made based upon the identified needs.

4. Recommendations and the way forward

Rapid sequencing of virus genomes is now achievable in varied settings, and analysis of SARS-CoV-2 genomic sequences has huge potential to inform public health efforts on COVID-19. The following is therefore recommended.

To WHO

1. Provide technical support to implement and enhance sequencing capacities in Afghanistan, Iraq, Lebanon, Libya, Somalia, Sudan, the Syrian Arab Republic and Yemen before the implementation of NGS in their national influenza centers.
2. Provide technical support to improve and strengthen the analysis and interpretation of virus genomic sequence data in these countries.



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