



Sequencing workshop Tuberculosis Nanopore

Workshop description.

Many of our sites in LMIC countries are conducting Mycobacterium tuberculosis (MTB) diagnostic testing, including drug sensitivity tests (DSTs), mainly using MGIT liquid culture, GeneXpert and HAIN assays. Whilst this is generally a successful way to identify MTB drug resistance and therefore ensure the correct regimen for a patient, multidrug resistant (MDR) and extensively drug resistant (XDR) MTB can often be difficult to treat. Patients with MDR and XDR TB are sometimes non-responsive to drugs, even when phenotypic DSTs or molecular tests suggest sensitivity, suggesting a greater complexity at the genetic level, or perhaps the presence of a non-tuberculous Mycobacterium (NTM) infection. In these cases of hard-to-treat infections, whole genome sequencing (WGS) may help to shine a light on the underlying causes and inform the clinical decision making process.

To conduct WGS, sites need to have access to a sequencing machine. The Illumina sequencing platform is often the first choice, especially for large-scale sequencing projects. However, in many of the LMIC sites, access to an Illumina MiSeq/HiSeq may not be possible; either there isn't such a facility within the country, or the scale is not appropriate (e.g. they only have very few samples they wish to sequence per month). Due to the large scale of the Illumina platforms, samples will be batched and so it can often take some time for results to be returned to the laboratory.

The Oxford Nanopore (ONT) MinION is a small, portable device that can be run from a laptop or desktop computer via the USB port within a local laboratory. ONT devices (such as the MinION) work by passing an ionic current through nanopores on a flow cell and measuring the changes in current as biological molecules (the DNA) pass through the nanopore or near it. The information about the change in current can be used to identify that molecule (e.g., A, T, G or C). The overlapping identical sections of these strings of bases can then be lined up, so that the whole genome can be predicted. This can then be compared to a reference genome and differences mapped. The MinION is designed to be run on 'any sample, by anyone, anywhere and provides a good, small scale option for laboratories to undertake WGS on isolates when it has previously proven difficult to identify the correct drug regimen.

The goal of the workshop

The goal of this workshop is to provide laboratory workers, who already have experience of working with TB, with the knowledge and practical experience to undertake WGS of TB samples using the ONT MinION system

The workshop is jointly organized by NIMR Muhimbili and University College London with funding from EDCTP.

General workshop information

Who can attend: Individuals with basic knowledge of TB and sequencing. Pre-course reading (TGHN tutorial videos, including bioinformatics, will be provided).

Location: National TB reference laboratory at Muhimbili Referral National Hospital, Dar es Salaam, Tanzania.

Fee: None. The project will cover all costs during the workshop, including laboratory costs, food and transportation. For those coming from abroad, visa fees, flights and accommodation will also be provided.

Application Requirements.

In order to ensure the course is tailored to the delegates knowledge and experience, please provide the supporting information to the table below and attach the following documents to the email:

- A current curriculum vitae (no more than 2 pages)
- Cover letter from the Employer confirming that you will be given time to participate in the training and related duties

Personal information	
Full name	
Gender	
Contact number (including country code)	
Email address	
Current employment	
Name of current employer	
Current employer address	
Current job title	
Start date	
End date	
Brief summary of main duties (250 words max)	
Reasons for attending (what would you like to get out of the course?) (250 words max)	
Any further requests related to the training course	
Relevant training courses attended (please provide details of courses you have attended or are currently attending)	
Please outline any knowledge or experience you have on TB diagnostics (250 words max)	
Please outline any knowledge or experience you have on sequencing (250 words max)	
Please outline any knowledge or experience you have on bioinformatics (250 words max)	
Current level of English language (please provide details of any qualifications you may have)	
Please rate your understanding of the following (1 being no experience/understanding and 10 being expert)	
TB culturing	1 2 3 4 5 6 7 8 9 10
TB DNA extraction	1 2 3 4 5 6 7 8 9 10
Oxford Nanopore library preparation	1 2 3 4 5 6 7 8 9 10
Linux (line command)	1 2 3 4 5 6 7 8 9 10
Sequencing data analysis (e.g. alignment)	1 2 3 4 5 6 7 8 9 10

Mode of selection; Participants will be selected based on the relevance of their application information.

Important dates

1. Advertisement released on 1st June 2022
2. Deadline for receiving applications 1st July 2022
3. Selection notification 15th July 2022. If you do not here from us by this date, consider yourself unsuccessful.
3. Course Dates 22nd – 25th August 2022.

Please send your application form and accompanying documentation to muhimbili@nimr.or.tz

Workshop schedule

Day	Session	Item
Day 1	Morning	Introduction to the pipeline Theory: optimising TB DNA extraction Theory: DNA quantification
	Afternoon	Laboratory practical: DNA extraction, part 1
Day 2	Morning	Laboratory practical: DNA extraction, part 2
	Afternoon	Laboratory practical: DNA quantification
Day 3	Morning	Theory: sequencing library preparation
	Afternoon	Laboratory practical: sequencing library preparation
Day 4	Morning	Theory: Introduction to sequencing bioinformatics (processing ONT data, QC, aligning, use of databases e.g. TB profiler)
	Afternoon	Practical: sequencing bioinformatics (with pre-prepared fast files)