FAQs

Q: Can we use one sequencing reagent for another machine?

A: Usually, the reagent kits for the sequencing reaction are platform-specific. Conversely, the library preparation kits offered by a given manufacturer can often be used across several different NGS platforms from that manufacturer. Most DNA-purification kits are compatible with different NGS systems.

Q: Is there common sequencing reagents used for RNA and DNA?

A: Usually, the reagent kits for the sequencing reaction step can be used for sequencing of DNA and cDNA. Library preparation kits can be different to facilitate the preparation of DNA or RNA for NGS.

Q: Could you explain more the minimum concentration of DNA?

A: The DNA input concentration is the platform-specific requirement range from a given manufacturer for efficient library preparation and sequencing reaction steps, e.g. Nextera XT DNA Library Preparation Kit (Illumina) recommended input is 1 ng.

Q: Among the different platforms, is Nanopore ideal for developing countries?

A: Surely, ONT sequencing (e.g. on MinION or Flongle) has many advantages in this regard: the capital investment is low; it is small and weighs less than 100 g; it is easy to operate where the experimental condition cannot be achieved; it does not require precise microscope alignment and repeated calibration; sample preparation can be inexpensive when using certain kits; it offers long reads and real-time analysis. However, challenges still exist, including being error prone

(low base-calling accuracy including indel) when compared to short reads sequencing.

Q: Are there already cost estimates for installation and implementation of WGS in low-income countries?

A: Not really. Cost effectiveness of the implementation of NGS in high burden settings for diagnostic and/or surveillance purposes has not been systematically investigated yet and represents a research need.

Q: BGI seq, how does DNA nanoball technology work?

A: The sequencing protocol utilized by BGI is called combinatorial probe-anchor synthesis (cPAS). This consists of rolling circle replication with the Phi 29 DNA polymerase, which synthesizes a long, single-stranded DNA that self-assembles into a nanoball (around 300 nanometres across). Fluorescent probes are incorporated, and the nanoballs are attached to a silicon wafer flow cell where they selectively bind to the positively charged material in a highly ordered pattern. The emission of fluorescence is then imaged and measured to record the base position.

Q: Illumina iSeq: 2-3 MTB genome/run - Is it concerning to the number of the sample per run?

A: Yes, when targeting around 100x reads coverage depth for WGS using the 2x150bp iSeq 100 i1 Reagent.

Q: I will really like to understand the term "high throughput"

A: High-throughput sequencing is the comprehensive term used to describe technologies that sequence DNA and RNA in a rapid and cost-effective manner. The distinction between "benchtop" and "high throughput" NGS instruments is made on the output achievable by a given instrument (volume of data) and it is in the range of few Mb to 15Gb for "benchtop" and up to 6,000Gb for "high throughput".

Q: Can one use the NanoDrop spectrophotometer to check DNA concentration or only Qubit Florometer is advisable?

A: Use a fluorometric-based method for an accurate and precise quantification of dsDNA (e.g. Qubit dsDNA HS). These dsDNA quantification kits enable quick and selective detection of low and high abundance DNA samples, and can distinguish dsDNA from ssDNA, RNA, protein, and free nucleotides. Avoid methods that measure total nucleic acid, such as NanoDrop or other UV absorbance methods.

Q: What are the differences between LRS and SRS?

A: The predominant difference between LRS and the conventional SRS approaches is the significant increase in read length. In contrast to short reads (150–300 bp), LRS has the capacity to sequence on average over 10 kb in one single read, thereby requiring less reads to cover the same genomic portion. Short reads are accurate, cheaper to produce (less reagents and cycles) and are completely adequate when you have a reference genome but long reads can improve de novo assembly, mapping certainty, transcript isoform identification, and detection of structural variants.

Q: What is the best device for NGS to implement in a low income and middle country?

A: The choice of methods (WGS - whole genome sequencing vs tNGS- target next generation sequencing) and NGS equipment depends among others on: the objectives of NGS use (detection of drug resistance for surveillance or patient care, molecular epidemiology, outbreak investigation, research…); placement; batch size; turnaround times; specimen referral systems; distributor availability; supply chain; capital investment; infrastructure requirements; sustainability.

Q: I would have liked more detail on targeted sequencing platforms

A: Targeted NGS (tNGS), as the name suggests, is best suited for applications in which one already knows the genes or gene regions to be investigated. tNGS assays require lower amounts of starting DNA, which in turn makes it possible to use tNGS approaches with pathogens recovered directly from clinical specimens. Any NGS platforms can potentially run tNGS protocols, provided differences in costs, batching and accuracy.

Q: Would be good to have a cost breakdown for newly implementation of NGS.

A: The upcoming "Practical considerations for implementing next-generation sequencing for drug-resistance surveillance in national TB programmes" implementation guide will list key considerations to guide budget development.

Q: Thermo fisher scientific technique of sequencing.

A: Ion Torrent next-generation sequencing exploits the fact that addition of a dNTP to a DNA polymer releases a hydrogen ion. The instrument measures the pH change resulting from those hydrogen ions using semiconductors, simultaneously measuring millions of such changes to determine the sequence of each fragment.

Q: How do you validate NGS for DR for pyrazinamide if the reference standard is unreliable?

A: Please refer to the information included in the WHO "Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance" and PMID: 35373160. As individual mutations associated with PZA resistance are both found less frequently and distributed more broadly among genes such as pncA than other resistance mutations (such as those in rpoB), they required special consideration. Therefore, a "relaxed" grading criteria was applied with less stringent thresholds to identify additional, infrequent mutations associated and not associated with resistance to PZA in the pncA gene only.

Q: Thresholds and group association.

A: From the WHO "Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance": Once the algorithm had identified variants associated with and not associated with resistant phenotypes, and relevant association statistics were generated as described in "Association studies" section, a set of consensus statistical thresholds and expert rules for confidence grading and ranking the observed MTBC mutations were applied to stratify the data into five groups according to the strength of the evidence for a genotype–phenotype association and the level of support for the phenotypic method used (see "Prioritization of phenotypic DST results" section). According to these grading criteria, the final mutation associations were stratified into five groups:

1. Associated with R
2. Associated with R – Interim
3. Uncertain significance
4. Not associated with R – Interim
5. Not associated with R

Q: Is there any recommendations on how to extract DNA from biopsy specimens etc?

A: There's no recommended procedure for DNA extraction and purification from specimens other than sputum samples and downstream NGS. However, researchers are trying to identify the best performing extraction procedures and a review of literature can help. Main challenge is the paucibacillary nature of such sample types.

Q: Examples of implementation of WGS in routine testing.

A: some manuscripts on this:

"Evaluating the clinical impact of routine whole genome sequencing in tuberculosis treatment decisions and the issue of isoniazid mono-resistance". BMC Infect Dis. 2022 Apr 7;22(1):349. doi: 10.1186/s12879-022-07329-y. PMID: 35392842.

"Whole-Genome Sequencing Has the Potential To Improve Treatment for Rifampicin-Resistant Tuberculosis in High-Burden Settings: a Retrospective Cohort Study. J Clin Microbiol. 2022 Mar 16;60(3):e0236221. doi: 10.1128/jcm.02362-21. Epub 2022 Mar 16. PMID: 35170980.

"Retrospective evaluation of routine whole genome sequencing of Mycobacterium tuberculosis at the Belgian National Reference Center, 2019". Acta Clin Belg. 2021 Nov 9:1-8. doi: 10.1080/17843286.2021.1999588. Epub ahead of print. PMID: 34751641.

"Mortality from drug-resistant tuberculosis in high-burden countries comparing routine drug susceptibility testing with whole-genome sequencing: a multicentre cohort study". Lancet Microbe. 2021 Jul;2(7):e320-e330. doi: 10.1016/S2666-5247(21)00044-6. Epub 2021 Apr 29. PMID: 35252901.

Q: In the algorithm for NGS, why is it required to collect new fresh specimens?

A: This is not always necessary as the sediment leftover from the culture sample may be used for DNA extraction and tNGS, depending on the algorithm adopted.

Q: Does the guidelines on use of NGS for TB developed in 2021, cancels the use of those developed in 2018 and 2020?

A: No, previous guidelines are usually updated with the new ones but necessarily overcome in all their contents.