



Next Generation Sequencing for COVID-19

Partnership for Supply Chain Management

Background

One of the pillars outlined by The Global Fund in the COVID-19 Response Mechanism is Surveillance, including Epidemiological Investigation and Contact Tracing. As part of the diagnostic implementation packages under this pillar, “Support for genomic surveillance / sequencing activities”, has been highlighted as an intervention that will contribute to the control of the COVID-19 pandemic. Therefore, increasing sequencing capacity and access to existing capacity across the world is a high priority in the fight against the pandemic.

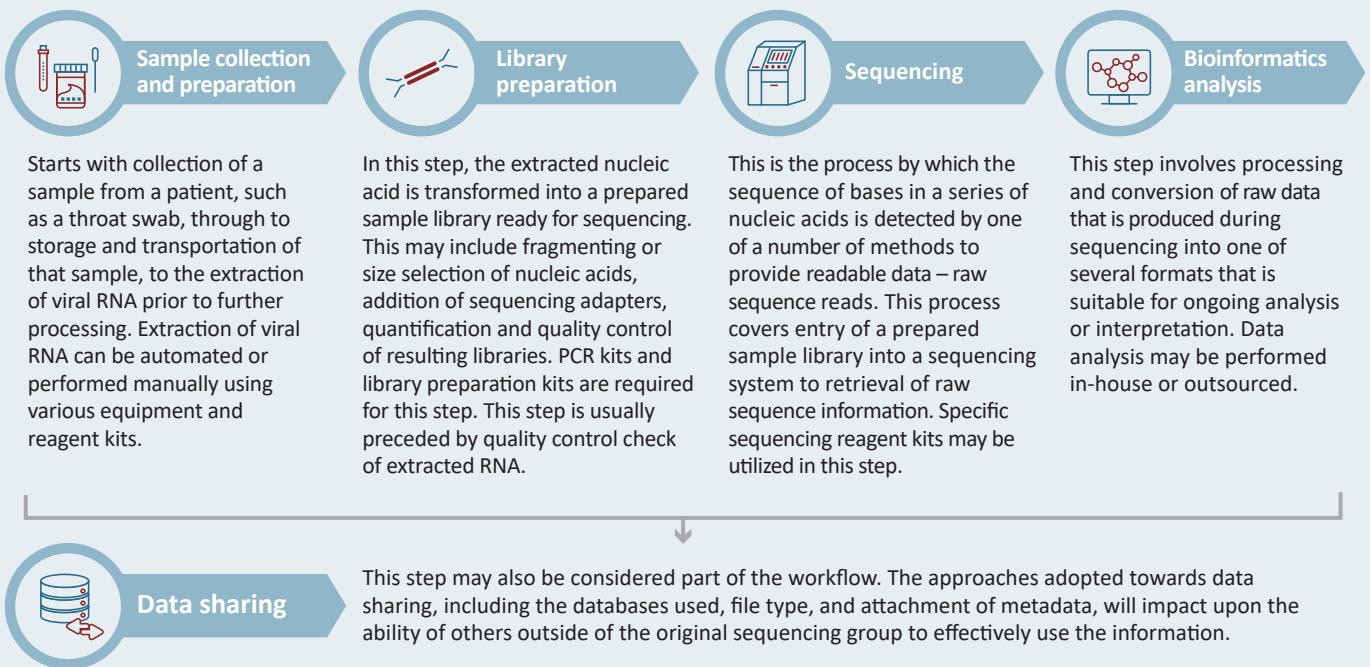
Next Generation Sequencing (NGS) is a high-throughput methodology that enables rapid sequencing of the base pairs in DNA or RNA strain samples. This technique provides an effective, unbiased and sensitive way to identify coronavirus mutations (new virus strains). NGS can be used in different areas, including, helping to track transmissions, studying viral genetics,

improving diagnostics and evaluating the efficacy of control measures. This increased recognition that NGS can contribute to improving public health is driving more laboratories to invest in this area. However, the cost and work involved are substantial, and laboratories need to have a clear idea of the expected public health returns on this investment. In addition, most NGS equipment and products are labelled as research use only and only few of them are can be used for diagnostic purposes.

General steps of the Next-generation sequencing workflow

NGS workflow design and choice depends on the specific needs of a project and resources available. Several steps are completed to produce readable genomic data from a collected sample. Each of the steps are important to ensure quality information is retrieved from the sample. The key steps in a basic workflow are outlined below:

GENERAL STEPS OF NGS WORKFLOW



5 Key considerations for implementation of NGS

There is a variety of technologies, methods, workflows and protocols for sequencing of SARS-CoV-2 and sequencing groups use what they prefer to generate the required data. Despite this variety, there are important factors to consider for successful implementation of NGS. Some of these are listed below:

- 1 **Technology throughput and turnaround times** – Analyzers available on the market for NGS differ in size and capacity. It is important to select appropriate analyzers for each sequencing project and ensure maximum utilization of the equipment.
- 2 **Availability of technical and equipment support** – NGS is a technique that involves use of specialized technical skills, analyzers and other equipment. Availability of installation, user training and equipment maintenance from the technology provider is important for smooth set up or expansion of NGS projects.
- 3 **Existing infrastructure and ancillary equipment** – It is important to assess already existing infrastructure such as PCR laboratories and ancillary equipment already available to ascertain additional requirements needed for NGS projects. A laboratory may make use of already existing equipment and infrastructure as NGS projects are considered.
- 4 **Cost** – NGS projects require significant investment. Investments associated with infrastructure, analyzers and equipment, reagents, sample collection and transportation, training and personnel, project logistics, data management and sharing, and all aspects of the NGS project should be considered.
- 5 **Data management and connectivity** – A clear strategy on how data generated from the NGS project will be collected, analyzed, stored and shared, including all hardware and software requirements needs to be in place.



Current list of NGS Platforms available through The Global Fund

Instrument	Manufacturer
iSeq100	Illumina
MiniSeq	Illumina
MiSeq	Illumina
NextSeq 550	Illumina
NextSeq 1000	Illumina
NextSeq 2000	Illumina
DNBSEQ G50	MGI
DNBSEQ G400	MGI
Ion GeneStudio™ S5 Prime System plus Ion Chef™ Instrument	Thermo Fisher Scientific
Ion Torrent Genexus™ Integrated Sequencer	Thermo Fisher Scientific

** List is not intended to be exhaustive and will be updated as other analyzers become available

Additional laboratory equipment required for the complete NGS workflow

Workflow element	Type of equipment required	Description
Extraction	Automated extraction instrument	There is a large variety of available commercial instruments for semi or fully automated extraction of nucleic acid from patient samples.
	Manual extraction	Most manual extraction kits do not require additional equipment other than those items listed below.
Liquid handling	Molecular grade pipettes	Several steps in the NGS workflow require accurate measuring of microliter volumes. Manual, single and multi-channel, and axial pipettes are commonly used. Liquid handling automated options are also available for high throughput laboratories.
RNA quantification	Spectrophotometer	Quantification of extracted nucleic acid is required at one or more stages of most NGS workflows. Any one or more of the listed types of instruments can be used.
	Fragment analyzer	
Amplification and incubation	Thermocycler	Almost all NGS workflows (both targeted and Whole Genome Sequencing) require one or more steps of amplification using reagents. There are many commercially available options.
Sample Purification	Magnetic stand	Many reasonably priced, commercially available options for separating different reagent components using magnetic beads.
	High-speed microplate shaker	For high-throughput reagent mixing.
	Centrifuge	Tube or plate centrifuges available, depending on workflow needs.
	Vortexes	For reagent mixing.
Sample and reagent storage	-20 Freezer	Any reliable, cost-effective freezer is suitable for storing samples and reagents.

References

- 1) The Global Fund COVID-19 Response Mechanism Information Note, The Global Fund
- 2) Next Generation Sequencing for SARS-CoV-2, Foundation for Innovative New Diagnostics
- 3) <https://www.thermofisher.com/nl/en/home/life-science/sequencing/next-generation-sequencing.html>
- 4) <https://www.illumina.com/products/by-type/ivd-products/covidseq.html>
- 5) <https://apps.who.int/iris/bitstream/handle/10665/274443/WHO-CDS-TB-2018.19-eng.pdf?sequence=1&isAllowed=y>