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

Sample collection and preparation

Starts with collection of a sample from a patient, such as a throat swab, through to storage and transportation of that sample, to the extraction of viral RNA prior to further processing. Extraction of viral RNA can be automated or performed manually using various equipment and reagent kits.

Library preparation

In this step, the extracted nucleic acid is transformed into a prepared sample library ready for sequencing. This may include fragmenting or size selection of nucleic acids, addition of sequencing adapters, quantification and quality control of resulting libraries. PCR kits and library preparation kits are required for this step. This step is usually preceded by quality control check of extracted RNA.

Sequencing

This is the process by which the sequence of bases in a series of nucleic acids is detected by one of a number of methods to provide readable data – raw sequence reads. This process covers entry of a prepared sample library into a sequencing system to retrieval of raw sequence information. Specific sequencing reagent kits may be utilized in this step.

Bioinformatics analysis

This step involves processing and conversion of raw data that is produced during sequencing into one of several formats that is suitable for ongoing analysis or interpretation. Data analysis may be performed in-house or outsourced.

Data sharing

This step may also be considered part of the workflow. The approaches adopted towards data sharing, including the databases used, file type, and attachment of metadata, will impact upon the ability of others outside of the original sequencing group to effectively use the information.

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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.

NGS equipment and reagent support availability by vendor

	Workflow Element					
Manufacturer	Extraction	Library preparation	Amplification	RNA quantification	Quality Control	Sequencing
Agilent		X			X	X
Applied Biosystems			X			
BioMerieux	X					
Biorad			X			
Hain	X		X			
Hongshi Medical			X			
Illumina		X				X
New England Biolabs		X				
Qiagen	X		X			
Roche	X		X			
Sansure Changsha	X					
Seegene	X					
Shanghai Kehua	X					
Shanghai ZJ Bio-Tech	X					
Thermofisher	X	X	X	X	X	X
Vela	X	X	X			X
Wantai	X					
Oxford Nanopore Technologies		X		X	X	X

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