

TB: THE NEXT GENERATION (SEQUENCING)

Applications for NGS Technologies in Tuberculosis Testing

Next generation sequencing (NGS) is a large-scale, high-throughput DNA sequencing technology that has powerful applications in the field of tuberculosis (TB) testing. This laboratory method can generate an enormous amount of data in one test—within hours to days—that can be used for a variety of purposes including:

- Comprehensive detection of mutations that predict drug resistance and susceptibility
- Identification of specific strains or species of an organism, such as *Mycobacterium tuberculosis* complex (MTBC)
- Comparison to historic data/genotyping
- High quality analysis to determine relatedness to other cases

During NGS, genomic DNA is sequenced many times and assembled by sophisticated software to form a consensus sequence.



NGS technology is used to perform:

Targeted NGS (tNGS), or amplicon-based NGS, to sequence specific parts of the genome, such as a single gene/loci or group of genes.

Whole genome sequencing (WGS) to sequence the entire genome of an organism.

Metagenomic sequencing to sequence all nucleic acid in a sample, including multiple organisms.

Choosing Between tNGS, WGS and Metagenomic Sequencing

Features	tNGS	WGS	Metagenomic Sequencing
Application	Useful for diagnostic purposes; can be used on primary specimens	Useful for diagnostics and epidemiological/surveillance purposes	Useful for diagnosis of polymicrobial infections or when diagnosis is difficult
Turnaround Time	2–5 days	4–7 days	~5 days
Cost Per Sample <i>Approximate; cost varies with testing volume.</i>	\$75–200	\$50–200	\$500
Considerations	<ul style="list-style-type: none"> • May require less data analysis • Generates data specific to the targets • More challenging assay design and wet lab processes 	<ul style="list-style-type: none"> • Generates large amounts of data, so analysis requires significant computational resources and expertise • Requires large amount of DNA/organism load 	<ul style="list-style-type: none"> • Massively parallel, deep sequencing of all material in a given sample • Complex and computationally intensive genomic data analysis

Resource Requirements & Considerations

Financial



Cost per sample, equipment maintenance, contracts, personnel

IT Infrastructure



Data reporting and storage, LIMS

Bioinformatics



Pipelines, cloud computing

Personnel



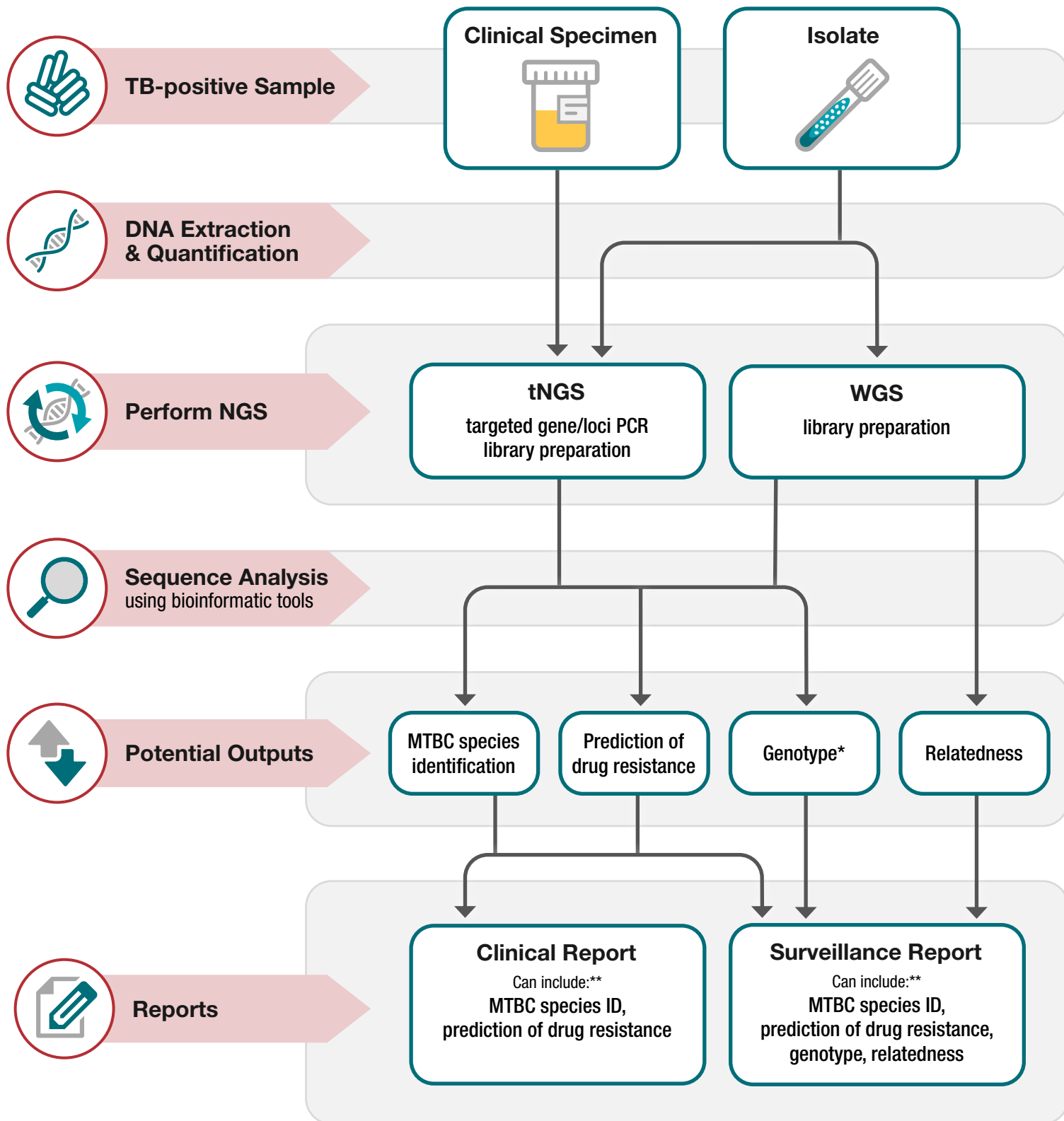
Qualified staff, training and competency

Instrumentation



Quality assurance, regular utilization, troubleshooting

TB NGS Workflow



* Limited genotypic information (e.g., lineage or spoligotype) with tNGS

** Outputs reported will depend on the sequencing method, who is receiving the report and what has been validated for clinical reporting