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

**Choosing Between tNGS, WGS and Metagenomic Sequencing**

<table>
<thead>
<tr>
<th>Features</th>
<th>tNGS</th>
<th>WGS</th>
<th>Metagenomic Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Application</strong></td>
<td>Useful for diagnostic purposes; can be used on primary specimens</td>
<td>Useful for diagnostics and epidemiological/surveillance purposes</td>
<td>Useful for diagnosis of polymicrobial infections or when diagnosis is difficult</td>
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<tr>
<td><strong>Turnaround Time</strong></td>
<td>2–5 days</td>
<td>4–7 days</td>
<td>~5 days</td>
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<tr>
<td><strong>Cost Per Sample</strong></td>
<td>Approximate; cost varies with testing volume</td>
<td>$75–200</td>
<td>$50–200</td>
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<tr>
<td><strong>Considerations</strong></td>
<td>• May require less data analysis</td>
<td>• Generates large amounts of data, so analysis requires significant computational resources and expertise</td>
<td>• Massively parallel, deep sequencing of all material in a given sample</td>
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<td></td>
<td>• Generates data specific to the targets</td>
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<td></td>
<td>• More challenging assay design and wet lab processes</td>
<td>• Requires large amount of DNA/organism load</td>
<td></td>
</tr>
</tbody>
</table>

**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

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.
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**TB NGS Workflow**

- **TB-positive Sample**
- **DNA Extraction & Quantification**
- **Perform NGS**
- **Sequence Analysis** using bioinformatic tools
- **Potential Outputs**
- **Reports**

**Clinical Specimen**

- **Isolate**

**tNGS**
- targeted gene/loci PCR
- library preparation

**WGS**
- library preparation

**Sequence Analysis**

- **MTBC species identification**
- **Prediction of drug resistance**
- **Genotype**
- **Relatedness**

**Potential Outputs**

- **Clinical Report**
  - Can include:**
  - MTBC species ID, prediction of drug resistance

- **Surveillance Report**
  - Can include:**
  - MTBC species ID, prediction of drug resistance, genotype, relatedness

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* 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.