RUO

For research use only -
For US Markets

TB NGS Assay

- **Resistance Prediction for 15 Anti-TB Drugs**
  Easily predict resistance-associated mutations in *Mycobacterium tuberculosis* complex (MTBC) gene targets using G2M's CliSeq Interpreter platform, which provides automated analysis & interpretation of sequencing data.

- **Genotyping and Spoligotyping of MTBC Strains**
  Identify the lineage, sub-lineage, and spoligotype of MTBC strains in the sample. Detect mixed infections involving distinct MTBC lineages or sublineages.

- **Identification of Over 100 Mycobacterial Species**
  Recognize mycobacteria of clinical or veterinary importance, including MTBC, *M. kansasii, M. abscessus, M. intracellulare, M. avium* complex, and many more. Detect co-infections or co-colonizations with distinct species.

- **Report in 2 Days**
  Save valuable time by extracting DNA directly from research samples, prepare libraries with easy to use protocol, sequencing on the Second Generation sequencing platform, and analyzing results in the CliSeq Interpreter platform.

Introduction

Efficient tuberculosis treatment relies on the rapid and early detection of drug resistance. In 2020, the World Health Organization reported over half a million new cases of rifampicin-resistant (RR) or multidrug-resistant (MDR) tuberculosis, including more than 25,000 cases classified as pre-extensively drug-resistant (pre-XDR) or extensively drug-resistant (XDR) forms[1]. Obtaining sequencing data of MTB by next-generation sequencing (NGS) technology holds a promise of improving the detection of drug resistance TB.

However, the routine use of whole-genome sequencing (WGS) remains constrained by the need for time-intensive mycobacterial culturing, while alternative molecular methods are limited to identifying a narrow set of common resistance-associated mutations[2,3].

The G2M TB NGS assay has been designed as a cost effective test with a fast turnaround time. This NGS-based targeted sequencing tool enables simultaneous prediction of resistance to 15 anti-tuberculosis drugs or drug classes, MTBC genotyping, and mycobacterial species identification. The assay can be used directly with research samples and the data can be analyzed and reported using G2M's Data analytics and reporting platform Cliseq Interpreter.

This assay is designed to map 53 kb region of the *M. tuberculosis* genome for 30 drug resistance genes and associated mutation sites, as well as SNP loci. It is a probed based panel specifically designed for the identification, genotyping, and antibiotic resistance prediction in genes and loci associated with *M. tuberculosis* complex (MTBC) while being compatiable with the commonly available sequencing platform (Illumina, MGI, AVITI).

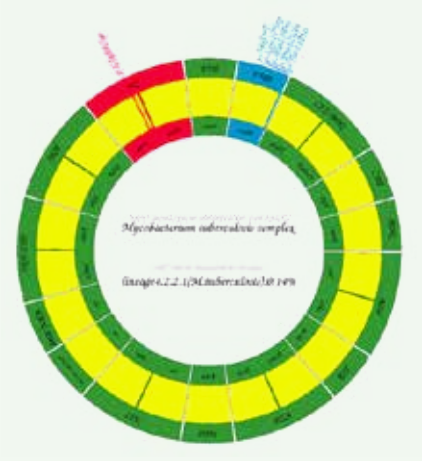


Figure 1 Identification genotyping and antibiotic resistance gene prediction of MTBC.
Red indicates the mutations related to antibiotic resistance. Blue indicates the presence of novel mutations within the targeted gene. Green indicates either the absence of mutations in the targeted gene or the presence of mutations unrelated to drug resistance.

End to End Workflow

The targeted libraries are constructed using G2M TB NGS Panel. The genomic DNA of 50-100ng extracted from sputum is subjected to fragmentation, end repaired and dA-Tailed. Further, adapter ligation is performed to attach sequencing technology specific adapters. The adapter ligated DNA is purified using MPB DNA Clean-up Beads. The adapter ligated DNA is indexed via indexing PCR and amplified for 5 cycles followed by purification using MPB DNA Clean-up Beads. Quantity and quality checks of libraries are performed. Further the libraries are pooled (4 plex) in equal concentration to set up a hybridization reaction for target capture for 4 hours. Target capture is performed using MSB streptavidin beads and then the on-bead library is PCR amplified for 14 cycles. Quantity and quality checks of libraries are performed. Sequencing can be performed as per the platform of choice (currently validated on Illumina, Element Biosciences (AVITI) and MGI platforms).

DNA extraction from research samples → **Library preparation & Hybridisation** → **Deep sequencing** → **Secure cloud based analysis** → **Visualisation Reporting**

Know beyond TB drug resistance

This comprehensive assay predicts resistance to 15 anti-tuberculosis drugs or drug classes, including newer compounds like Bedaquiline and Linezolid, making it one of the most extensive genotypic tool directly applicable to research specimens. This assay also aids in the identification of MTBC strain types within a sample. MTBC strains are spoligo-typed and genotyped based on nucleotide identity of the hsp65 gene.

Cliseq Interpreter platform helps in analysing the data and reporting the information using an illustratitive format (as shown in the figure 1). The panel ensures 100% gene coverage at 5000X depth.

First-line drugs

rpoB	Rifampicin
ahpC, fabG1, katG, inhA	Isoniazid
pncA⁺	Pyrazinamide
embB	Ethambutol

Identification

hsp65	Species ID
CRISPR/ DR	Spoligotyping
phyloSNPS	Genotyping

Group A/B (XDR)

Fluoroquinolones	*gyrA, gyrB*
Bedaquiline, Clofazimine	*rv0678*⁺
Linezolid	*rrl, rplC*

Group C

Amikacin	*rrs*
Streptomycin	*gidB, rrs, rpsL*⁺
Ethionamide	*ethA*⁺*, inhA, fabG1*

Other

Capreomycin	*tlyA*⁺*, rrs*
Kanamycin	*eis, rrs*

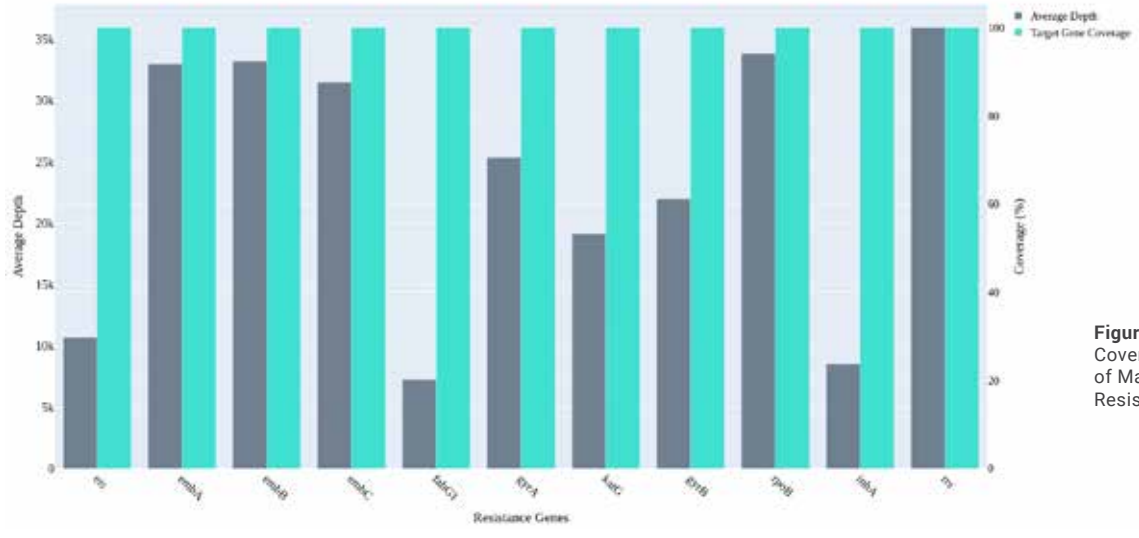


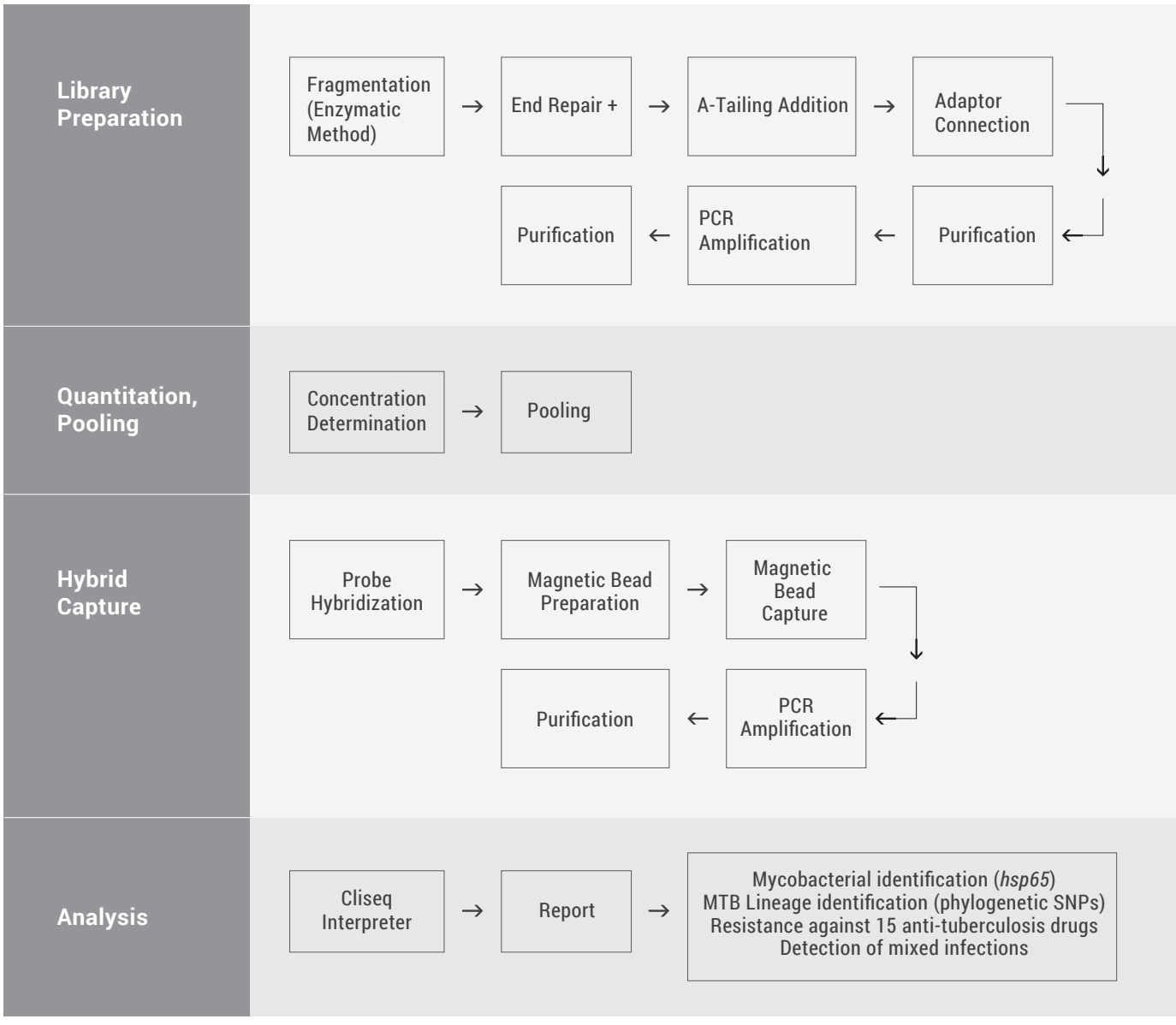
Figure 2B. Coverage Depth of Major Drug Resistance Genes

Rapid time to result

The G2M TB NGS assay eliminates the need for mycobacterial cultures and can be applied directly to research samples with even minimal bacterial loads. The entire process from DNA extraction, followed by genomic library preparation, hybridisation and sequencing preparation takes 2 to 3 days. Post sequencing, FASTQ (read) files can be uploaded to our cloud based platform using the coupon credentials provided along with the kit. The fully automated reporting pipeline analyses the data in under an hour, with helps in graphic-visualization reporting.

Input sample type	gDNA from research samples - sputum/culture/BAL
DNA input quantity	50 - 100 ng
Library preperation	G2M TB NGS
Recommended sequencing technologies	Illumina®, MGI, Element Biosciences
Turnaround time	48 hours
Storage & shelf-life	-20°C for up to a year

G2M TB NGS Workflow



References

1. World Health Organisation, Global tuberculosis report. 2021.
2. Rahman, A. et al. Comparison of Xpert MTB/RIF assay and genotype MTBDRplus DNA probes for detection of mutations associated with rifampicin resistance in Mycobacterium tuberculosis. PLoS One 11, 1–11 (2016).
3. Rufai, S. B. et al. Comparison of xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant Mycobacterium tuberculosis. J. Clin. Microbiol. 52, 1846–1852 (2014).

Scan for Sample Report

ISO 13485:2016 | ISO 9001:2015 | GMP Quality

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