Deeplex® Myc-TB

From clinical samples to extensive drug resistance profile

Culture-free prediction of tuberculosis drug resistance

Recommended by WHO for rapid, extensive diagnosis

GenoScreen

Deep sequencing-based assay for extensive antibiotic resistance prediction of *Mycobacterium tuberculosis* complex, with mycobacterial identification and genotyping

Highlights

- **Prediction of resistance to 13 anti-TB drugs**
  Easily visualise resistance associated mutations in (detected) *M. tuberculosis* complex (MTBC) gene targets, thanks to our Deeplex web app for automated analysis and interpretation of the sequencing data.

- **Genotyping and spoligotyping of MTBC strains**
  Get to know the lineage / sublineage and spoligotype of MTBC strains present in the sample. Detect mixed infection involving distinct MTBC lineages/sublineages.

- **Identification of more than 100 mycobacterial species**
  Identify mycobacteria including most species of clinical or veterinary relevance: MTBC*, M. kansasii, M. abscessus, M. intracellulare, M. avium complex,* and many more. Detect co-infection/co-colonization with distinct species.

- **Turnaround time of less than 48 hours**
  Save time using DNA from clinical samples[a] prepare libraries, sequence and analyse results in the Deeplex web app for a total turnaround time of less than 48 hours.

- **High performances**
  Mean sensitivity of 95% and mean specificity of 99% *vs* phenotypic drug susceptibility testing, **identify heteroresistance down to 3%** subpopulations and work with DNA loads down to 100 genomes.

Introduction

According to the World Health Organization (WHO), in 2023 there was an estimated 400 000 new cases of rifampicin (RR) or multi- drug (MDR) resistant forms, among which 29,000 tested pre- extensively drug resistant (pre-XDR)[b] or extensively drug resistant (XDR)[c]1. Rapid and accurate drug susceptibility testing (DST) is essential to treat tuberculosis (TB) efficiently.

Phenotypic DST is slow, and conventional molecular tests interrogate only a restricted number of resistance targets.
Here, we present the Deeplex® Myc-TB[2] assay that uses targeted next-generation sequencing (tNGS) for rapid simultaneous prediction of (hetero)resistance to 13 anti-tuberculosis drugs/drug classes, MTBC genotyping and mycobacterial identification. This comprehensive assay is applicable on DNA extracts from clinical samples[a].
The assay includes an automated pipeline and integrated databases[d] for analysis and interpretation of the sequencing data, using a secure web app (Figure1). Based on evaluation of clinical performance, implementability and cost-effectiveness across multiple settings, Deeplex® Myc-TB is uniquely recommended by WHO[3] for follow-on detection of resistance to rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, amikacin, streptomycin, linezolid, clofazimine and bedaquiline, both in drug-susceptible TB or MDR/RR-TB patients.

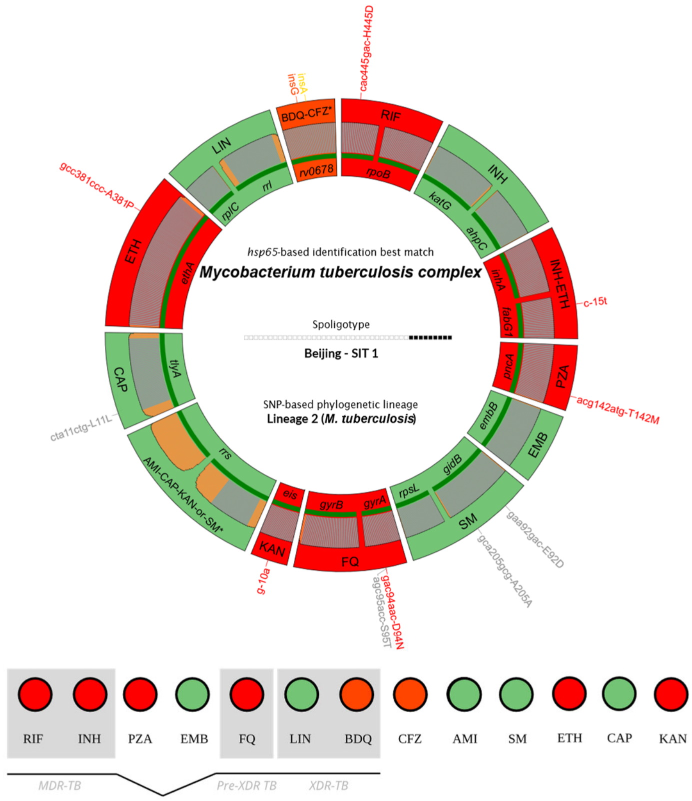


Figure 1. The Deeplex® web app (Top) **Deeplex® map** showing mutations associated (red) or not associated (or synonym, grey) with antibiotic resistance of MTBC. Information on mycobacterial identification is shown in the center of the map. (Bottom) **Resistotype** of an identified MTBC strain showing its predicted resistance pattern to 13 anti- tuberculosis drugs. The Deeplex® map is a registered design.

Rapid, extensive drug resistance prediction

Deeplex® Myc-TB testing starts from DNA extracted from clinical specimens from patients with bacteriologically confirmed TB, with other bacteriologically confirmed mycobacterial disease, or from mycobacteria-positive culture. A single multiplex PCR is then performed to amplify 18 MTBC gene targets associated with resistance to first- and second-line drugs (Figure 3), the *hsp65* gene (for mycobacterial speciation) and the DR/CRISPR locus (for spoligotyping).

This is followed by Illumina DNA library preparation and deep amplicon sequencing. The obtained sequencing data are then uploaded to a secure web app for automated analysis, user-friendly visualization and interpretation of sequencing data (Figure 2).

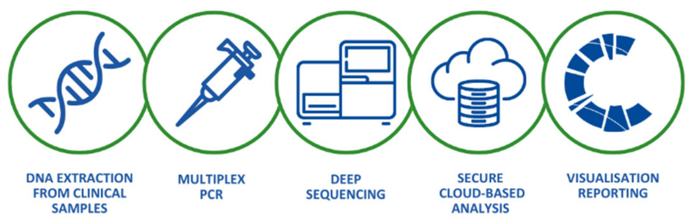


Figure 2. The Deeplex® Myc-TB workflow. From DNA extraction from clinical or culture samples to data analysis and result visualization.

Prediction of resistance to 13 anti-TB drugs

Based on detected presence or absence of resistance associated mutations in the targets, the MTBC-containing sample is predicted to be susceptible or resistant to each antibiotic (Figure 1). Results of variant calling and information on association or non-association with drug resistance or susceptibility can be easily visualized along with information on sequencing metrics for each target. Predicted susceptibility or resistance profiles can be readily viewed in the Deeplex resistotype and Deeplex map (Figure 1).

MTBC strain genotyping

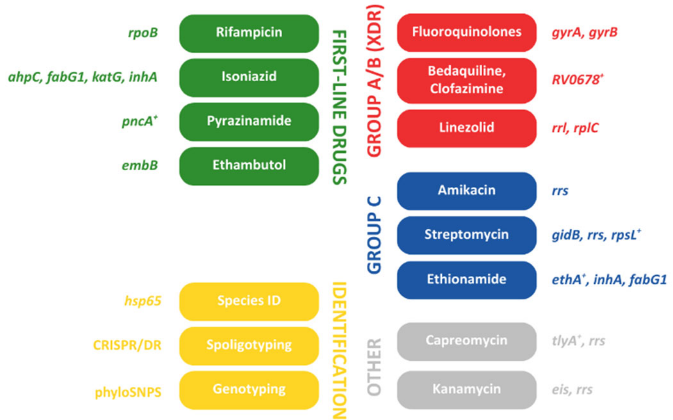


Figure 3. Genes or genes regions amplified and sequenced via the Deeplex® Myc-TB assay (* : full genes).

Deeplex® Myc-TB testing simultaneously identifies MTBC strain genotypes. Genotyping is achieved by detection of presence/absence of 43 reference spacers in the DR/CRISPR locus and phylogenetic SNPs in other gene targets, allowing to identify strain spoligotypes and MTBC (sub-)lineages. Genotyping results are represented in the center of the obtained Deeplex® map (Figure 1).

A highly sensitive assay

With the **Deeplex® Myc-TB** assay, sequencing of mycobacterial gene targets can be achieved at high read depth. This deep coverage ensures highly confident variant detection, even for heteroresistant subpopulations representing as little as 3% of the bacteria in a sample — beyond the detection limits of other rapid molecular assays. Extracted DNA representing as low as ~100 mycobacterial genomes, thus below microscopy positive grades, can be analysed. The **Deeplex® Myc-TB** assay has a weighted sensitivity of 95% and specificity of 99% vs a composite reference of MGIT phenotypic drug susceptibility testing and whole-genome sequencing for 13 anti-TB drugs/drug classes[4].

Figure 4. The Deeplex® Myc-TB kit includes a master mix ready for multiplexed amplification, a positive and internal DNA control as well as an activation code to access the Deeplex® web app.

Identification of more than 100 non-tuberculous mycobacterial species

Based on nucleotide identity of the *hsp65* gene[5], the **Deeplex® Myc-TB** assay can not only identify *M. tuberculosis* complex but also >100 other mycobacterial species, including most clinically relevant species such as *M. kansasii, M. intracellulare, M. avium* complex, *M. abscessus, M. chelonae, etc.*

Time to result of less than 48 hours starting from clinical sample

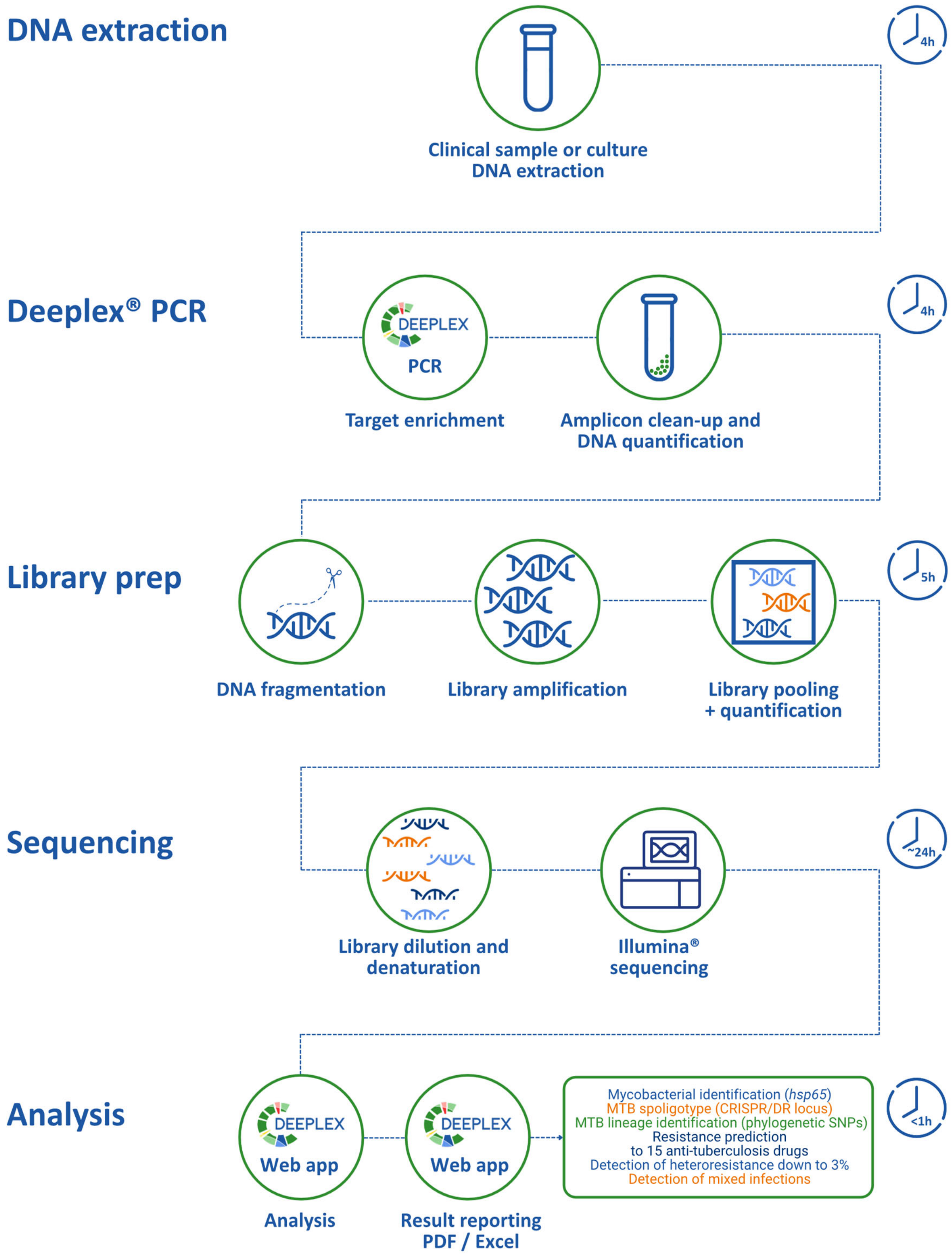
Mycobacterial cultures are not required for use with the **Deeplex® Myc-TB**, and the assay can be used on clinical samples with minimal bacterial loads (see above). The total workflow starting from DNA extraction takes 1-2 days depending on the Illumina sequencing platform used (Table 2). Upon completion of sequencing, output FASTQ (read) files can be directly uploaded onto our secure web app, using the access code provided within the kit. Data are analyzed with our fully parameterized Deeplex® pipeline and can be visualized in less than an hour.

Specifications	
Input sample type	gDNA from clinical samples[a] (eg. sputum...) or culture
Input sample concentration/volume	9 µl gDNA at 1 pg/µl mycobacterial DNA, 20 µL culture thermolysate, 200 µL sputum
Recommended library prep	Nextera® XT (Illumina®), Illumina® DNA prep
Recommended sequencing technologies	Illumina® iSeq 100 (13 samples), MiniSeq (21), MiSeq (45), NextSeq 550 (372)†
Turnaround time	iSeq 100: 1 day; others sequencers: ≈2 days
Storage and shelf-life	-20°C for up to a year
Formats and versions	RUO: 16 and 48 reactions CE-IVD: 48 reactions

Table 2. Specifications of the Deeplex® Myc-TB kit.
Turnaround time includes multiplex PCR, library preparation, sequencing and analysis. †Number of samples – controls not included.

[a] with genome loads ≥ 100
[b] MDR additionally resistant to fluoroquinolones.
[c] MDR additionally resistant to fluoroquinolones and, at least one additional group A drug (bedaquiline, linezolid).
[d] © 2025 GenoScreen Mycobacterium tuberculosis variant database (all rights reserved) and © Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance.

Deeplex® Myc-TB workflow



References

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2. Jouet A, Gaudin C, Badalato N, et al. Deep amplicon sequencing for culture6 free prediction of susceptibility or resistance to 13 anti-tuberculous drugs. Eur Respir J. 2021; 57(3):2002338.
3. WHO operational handbook on tuberculosis. Module 3: diagnosis. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO
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