DEEPLEX

Deeplex® Myc-TB

From clinical samples to drug resistance profile

A novel *Mycobacterium tuberculosis* drug resistance prediction assay, comprehensive, culture-free and based on deep sequencing

RESEARCH USE ONLY

GenoScreen
Innovative Genomics

A novel deep sequencing-based assay for antibiotic resistance prediction of *Mycobacterium tuberculosis* complex, with mycobacterial identification and genotyping

Highlights

- **Prediction of resistance to 15 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*, prepare libraries, sequence and analyse results in the Deeplex web app for a total turnaround time of less than 48 hours.

- **High performances**

  Capture 97-99% of resistance phenotypes predicted by WGS, mean sensitivity of 95.2% and mean specificity of 97.1% vs phenotypic drug susceptibility testing**, **identify heteroresistance down to 1%** subpopulations and work with DNA loads down to 100 genomes.

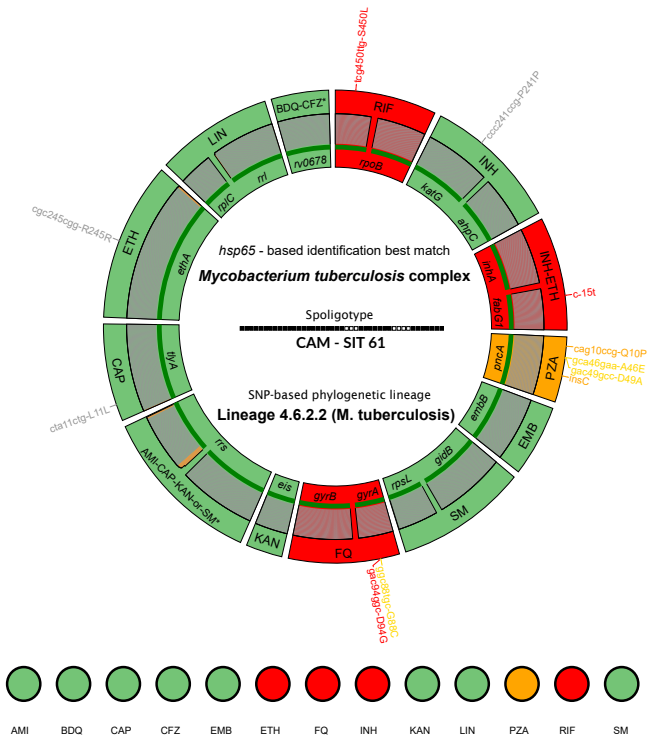


Figure 1. The Deeplex® web app (Top) **Deeplex® map** showing mutations associated (red and orange (heteroresistance)) or not associated (or synonym, grey) with antibiotic resistance of MTBC. Information on mycobacterial identification is shown in the center of the map. (Bottom) **Resistotype** of an identified MTBC strain showing its predicted resistance pattern to 15 anti-tuberculosis drugs. The Deeplex® map is a registred design.

Introduction

According to the World Health Organization, in 2020 there were more than half a million new cases of rifampicin (RR) or multi-drug (MDR) resistant forms, including more than 25,000 pre-extensively drug resistant (pre-XDR)*** or extensively drug resistant (XDR)**** forms[1]. Yet to treat tuberculosis efficiently, rapid and early detection of drug resistance is essential.

Advances in next-generation sequencing (NGS) technology, offer great potential for more efficient detection of drug resistant TB. Unfortunately today, routine clinical use of whole genome sequencing (WGS) requires time-consuming mycobacterial culturing and alternative molecular methods rely on a small set of common resistance associated mutations, limiting the detection spectrum[2,3].

Here, we present the **Deeplex® Myc-TB** assay which uses NGS-based targeted deep sequencing for the simultaneous prediction of (hetero)resistance to 15 anti-tuberculosis drugs/drug classes, MTBC genotyping and mycobacterial identification. This all-in-one assay is compatible with detection directly from clinical samples* and includes an automated analysis pipeline of the sequencing data in a secure web app with integrated databases (Figure1).

A comprehensive assay based on targeted sequencing

The **Deeplex® Myc-TB** assay starts with DNA extraction from either a (suspected) mycobacteria-containing clinical specimen or a mycobacteria-positive culture. A single multiplex PCR is then performed to amplify mycobacterial genome regions from 18 drug resistance associated MTBC genes, the *hsp65* gene (for mycobacterial speciation) and the DR/CRISPR locus of the MTBC (for spoligotyping).

The resulting PCR products are cleaned-up and libraries are prepared for sequencing. The obtained sequencing data are then uploaded to a secure web app for automated analysis, results can be viewed directly from the web app and exported in several formats (Figure 2).

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. Alternatively, the **Deeplex® Myc-TB** assay comes as a service (on demand). GenoScreen performs all steps, from DNA extraction (optional) to final generation of analysed data, made available to the user in the web app.

The assay has successfully been tested using the Nextera XT and Illumina DNA prep library kits on the iSeq 100, MiniSeq, MiSeq, and NextSeq 550 sequencing platforms (Illumina).

Prediction of resistance to 15 anti-TB drugs

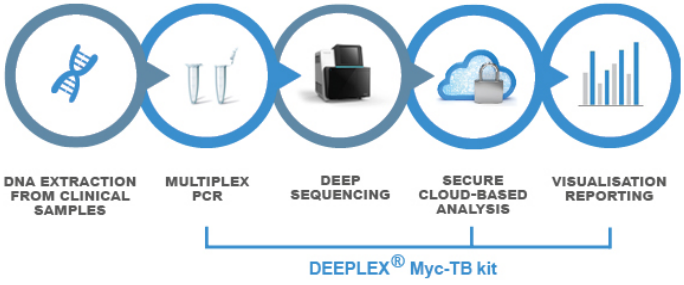


Figure 2. The Deeplex® Myc-TB workflow. From DNA extraction from clinical or culture samples to data analysis and result visualization. The assay comes as two options: the **Deeplex® kit** and the **Deeplex® service**. The kit includes a single PCR master mix ready for multiplex amplification of the mycobacterial targets, positive and internal control as well as an activation code to access the Deeplex® Web App. Service is performed at GenoScreen.

The **Deeplex® Myc-TB** assay relies on deep sequencing of 18 main MTBC gene targets associated with resistance to first and second line drugs (Figure 3). Based on the observed absence or presence of mutations in these loci and interrogation of reference databases*****, the MTBC strain present in the sample is predicted to be susceptible or resistant to each antibiotic, or with yet-to-be characterized mutations (Figure 1). Individual target positions and mutations can be easily visualized along with their sequence coverage depths. Information on reference literature describing the association of mutations with drug resistance can be accessed via hyperlinks. In total, the assay can predict resistance to 15 anti-tuberculosis drugs/ drug classes including the more recently introduced compounds such as bedaquiline and linezolid, making it the most exhaustive genotypic assay directly applicable on specimens available to date.

MTBC genotyping and spoligotyping

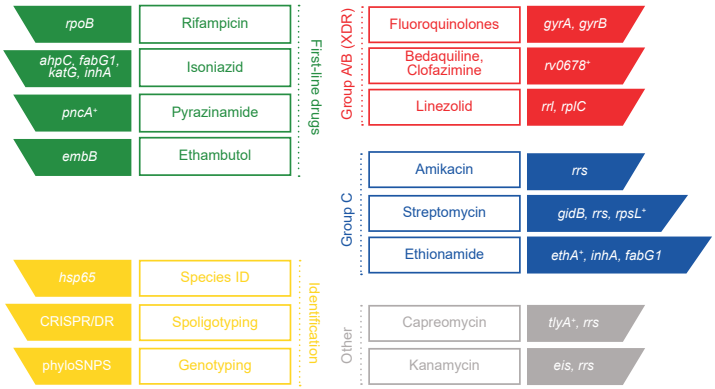


Figure 3. Genes or genes regions amplified and sequenced via the Deeplex® Myc-TB assay (+ : full genes).

In addition to antibiotic resistance prediction, the **Deeplex® Myc-TB** assay can be used to identify MTBC strain types present in the sample. When detected based on nucleotide identity of the *hsp65* gene, MTBC strains are spoligotyped and genotyped. This is achieved by detecting the presence-absence pattern of 43 spacers in the CRISPR locus and phylogenetic SNPs in the other gene targets, respectively. Mycobacterial species identification as well as MTBC spoligotyping and genotyping results can then easily be viewed on the Deeplex® web app, in the center of the 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 which means that each sequence position is covered by many reads, enabling highly confident mutation calls including for mutant/heteroresistant subpopulations representing as low as 1-3% of bacteria in the sample[4], inaccessible to other rapid molecular tests. Extracted DNA representing as low as 100 mycobacterial genomes, thus below the limit of detection by classical microscopy[4], can be characterized. The **Deeplex® Myc-TB** assay captures ***in silico*** 97-99% of resistance phenotypes predicted by WGS and has a mean sensitivity of 95.2% and a mean specificity of 97.1% vs phenotypic drug susceptibility testing**.

Drug	Sensitivity (%)	Specificity (%)
Rifampicin	99.4	98.8
Isoniazid	98.3	98.4
Pyrazinamide	85.7	100
Ethambutol	92.2	90.7
Streptomycin	90.7	98.9
Fluoroquinolones	91.7	99.2
Amikacin	100	100
Kanamycin	88.9	100
Capreomycin	93.8	97.4
Ethionamide	92.6	68
Linezolid	NA	100
Total	95.2	97.1

Table 1. Deeplex® Myc-TB phenotype predictions versus pDST
Comparaison of Deeplex® Myc-TB drug susceptibility and drug resistance predictions (excluding uncharacterized mutations) against pDST on a reference collection of 429 MTB isolates, including the WHO-TDR collection. The dataset used did not include any linezolid resistant strains (only linezolid susceptible strains, all correctly predicted as such by Deeplex®) and bedaquiline susceptibility was not tested. Deeplex® Myc-TB phenotype predictions obtained with Deeplex® Myc-TB web app version 1.4. **

Identification of more than 100 mycobacterial species

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

Turnaround time of less than 48 hours

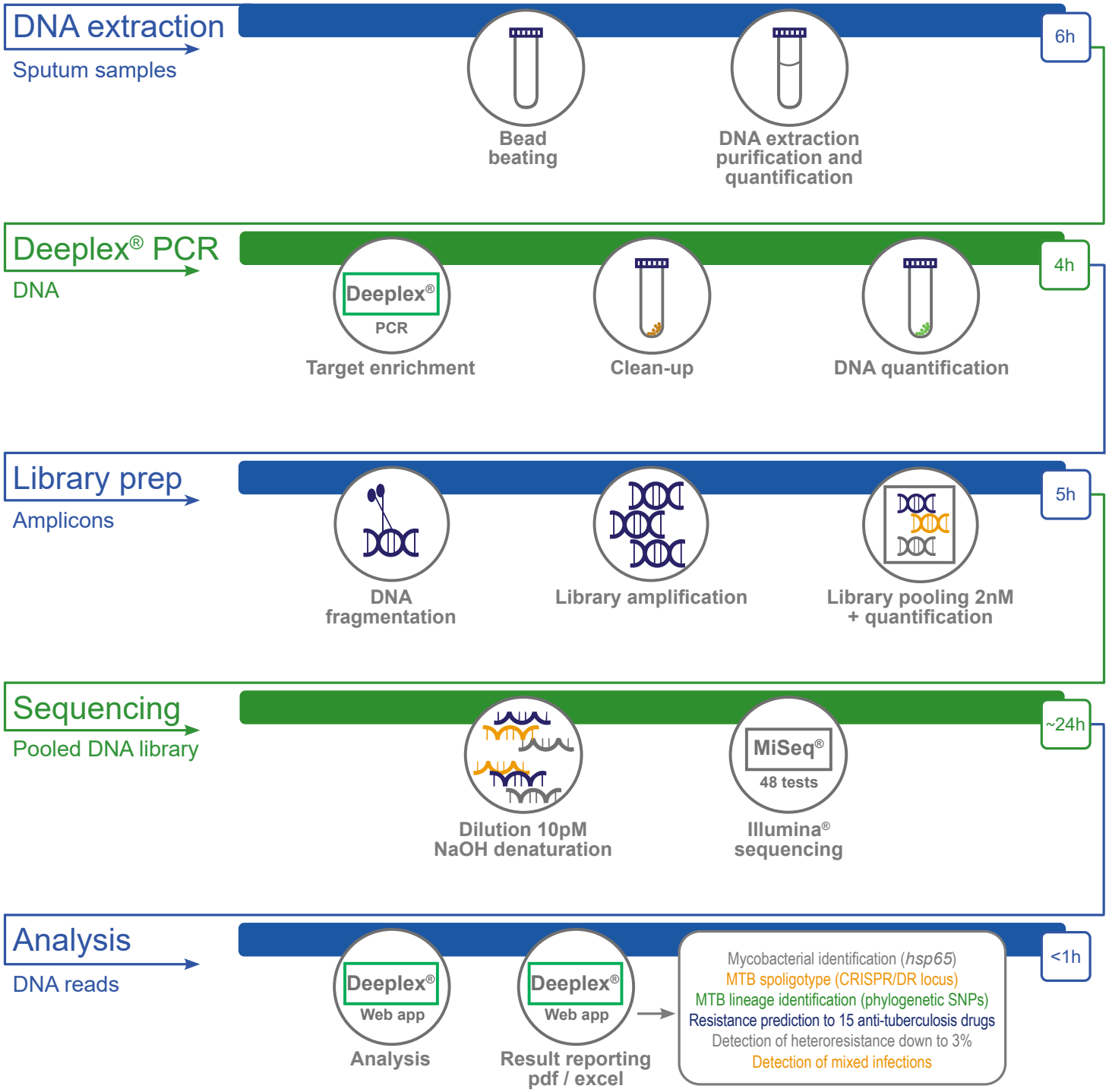
Mycobacterial cultures are not required for use with the **Deeplex® Myc-TB** and the assay can be used on clinical samples with minimal bacterial loads (see above). Deeplex® targets are amplified from extracted DNA with the ready-to-use master mix for multiplex PCR, purified and prepared to be sequenced. The total takes from 1 to 2 days (Table 2). Once targets are sequenced, output FASTQ (read) files are ready to be uploaded onto our secure web app, using the access code provided within the kit. Data will be analyzed with our fully parameterized Deeplex® pipeline in less than an hour and results can be easily visualized.

	Deeplex® Myc-TB
Input sample type	gDNA from clinical samples* (eg. sputum...) or culture
DNA input quantity	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)ᵗ
Turnaround time	iSeq 100: 1 day ; others sequencers: ≈2 days
Storage and shelf-life	-20°C for up to a year

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

** with genome loads ≥ 100*
*** Results from 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. Results were obtained on 109 clinical specimens directly analyzed by Deeplex® Myc-TB versus WGS on culture and on 429 MTBC strains versus phenotypic DST.*
**** MDR additionally resistant to fluoroquinolones.*
***** MDR additionally resistant to fluoroquinolones and, at least one additional group A drug (bedaquiline, linezolid).*
****** © 2022 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



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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).
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HEADQUARTERS:
1 rue du Pr. Calmette
59 000 Lille - FRANCE
Phone : +33(0) 362 263 777

CUSTOMER RELATIONS:
commercial.dept@genoscreen.com
Phone : +33(0) 362 263 776

FIND OUT MORE ABOUT GENOSCREEN:
www.genoscreen.com

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