GenoScreen
Innovative Genomics

Deeplex® Myc-TB

From tuberculosis clinical samples to drug resistance profile

A new *Mycobacterium tuberculosis* drug resistance prediction assay,

better, faster and culture-free, based on deep sequencing

DEEPLEX

A fast, deep sequencing-based assay for mycobacterial identification and genotyping + antibiotic resistance prediction of *Mycobacterium tuberculosis* complex (MTBC)

Highlights

- **Prediction of resistance to 15 antibiotics**

  Easily visualise mutations at loci involved in antibiotic resistance when MTBC is present, thanks to our automated analysis and Deeplex web app.

- **Genotyping and spoligotyping of MTBC strains**

  Get to know the lineage and spoligotype of *M. tuberculosis* complex strains when present in the sample.

- **Identification of 156 mycobacterial species**

  Identify mycobacteria including species pathogenic to humans and animals: *M. tuberculosis complex, M. leprae, M. kansasii, M. ulcerans*…

- **Turn-around time of two days**

  Save time using DNA from complex clinical samples, prepare libraries, sequence and analyse in the Deeplex web app in less than an hour, for a total turn-around time of ~2 days.

- **Highly sensitive**

  Characterize >98% of variants known to be involved in MTBC antibiotic resistance, identify minority populations of MTBC when present and work with low grade mycobacteria.

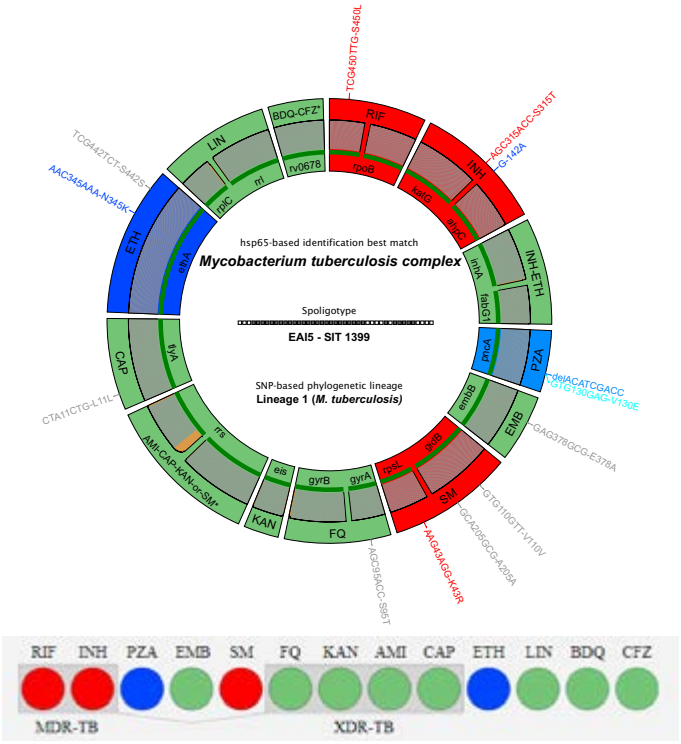


Figure 1. The Deeplex web app (Top) **Deeplex® map** showing mutations associated (red) or unassociated (or synonym, grey) with antibiotic resistance of MTBC along with yet-to-be characterized mutations (blue). Information on mycobacterial identification is shown in the center of the map. (Bottom) **Resistotype** of the identified MTBC strain showing resistance pattern to 15 antibiotics.

Introduction

According to the World Health Organization, only 160,684 out of the 330,000 estimated cases of drug-resistant tuberculosis were detected in 2017[1]. Yet to treat tuberculosis efficiently, rapid and early detection of the disease and of drug resistance is essential.

With increasing advances in next-generation sequencing (NGS) technology, development of novel, more efficient forms of diagnosis for tuberculosis is tempting. Unfortunately today, the use of whole genome sequencing (WGS) requires time-consuming mycobacterial culturing and alternative molecular methods rely on a small set of common mutations, limiting the detection of drug resistance[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 antibiotics, MTBC genotyping and mycobacterial identification. This all-in-one assay is compatible with the analysis of complex clinical samples and includes an automated sample analysis in a secure web app with integrated databases (Figure1).

An all-in-one assay based on targeted sequencing

The Deeplex® Myc-TB assay starts with the extraction of DNA from either a suspected mycobacterial sample or an MTBC strain culture. A multiplex PCR is then performed to amplify 22 mycobacterial loci. The resulting PCR product is cleaned-up and libraries are prepared to allow sequencing. Sequencing data is finally uploaded to a secure web app for automated analysis, results can be viewed directly from the web app or exported into several formats (Figure 2).

The assay comes in two flavours: the service and the kit. When using the Deeplex® Myc-TB service, GenoScreen performs all steps, from DNA extraction (optional) to data analysis. Else, the kit includes a master mix ready for amplification of 22 mycobacterial loci, DNA for a positive and internal control as well as an activation code to access the Deeplex web app prior to analysis.

The protocol has been tested using the Nextera XT library preparation kit and the MiniSeq, MiSeq, and NextSeq Illumina sequencing platforms.

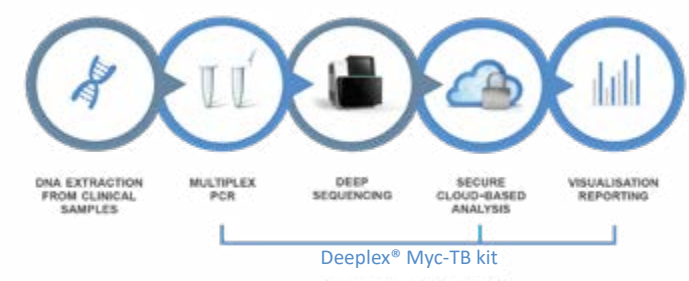


Figure 2. The Deeplex® Myc-TB workflow.
From DNA extraction from clinical or culture samples to data analysis and result visualization. The assay comes in two flavors: the **Deeplex kit** and the **Deeplex service**. The kit includes a master mix ready for amplification of 22 mycobacterial loci, DNA for a positive and internal control as well as an activation code to access the Deeplex Web App prior to analysis. Service is performed at GenoScreen.

Prediction of resistance to 15 antibiotics

The Deeplex® Myc-TB assay involves the amplification and sequencing of 22 mycobacterial loci including 20 (18 genes) known to be involved in MTBC resistance to first and second line drugs (Figure 3). Based on observed mutations at these loci and interrogation of available databases (Figure 4), a decision is made whether the MTBC present in the sample is susceptible, resistant or potentially resistant to each antibiotic. Mutations associated or unassociated (or synonym) with antibiotic resistance plus yet-to-be characterized mutations can easily be visualized in the Deeplex web app (red, grey and blue). In total, the assay can predict resistance to 15 antibiotics including the more recently introduced bedaquiline, clofazimine and linezolid[4], making it the most exhaustive genotypic assay to date.

Figure 3. Genes amplified and sequenced via the Deeplex® Myc-TB assay, enabling **mycobacterial identification** and **MTBC genotyping, spoligotyping** (green) and prediction of **resistance to first line** (dark blue) and **second line** (light blue) **antibiotics**.

MTBC genotyping and spoligotyping

In addition to antibiotic resistance prediction, the Deeplex® Myc-TB assay can be used to characterize MTBC strains present in the sample. When detected based on nucleotide identity at the *hsp65* gene, MTBC strains are spoligotyped and genotyped. This is achieved using the presence-absence pattern of 43 direct repeats at the CRISPR locus and phylogenetic SNPs at sequenced loci, respectively. Mycobacterial species identification as well as MTBC spoligotyping and genotyping results can then easily be viewed on the Deeplex web app, either at the center or on the right of the Deeplex map.

A highly sensitive assay

With the Deeplex® Myc-TB assay, sequencing of mycobacterial loci is achieved at high read depth which means that loci are sequenced many times, enabling both the detection of mutations with high confidence and the characterization of low grade mycobacteria. That is, population sizes as low as 100 genomes, left undetected by classical microscopy[5]. Deep sequencing also allows the identification of rare mutations, carried by down to 3% of bacteria in the sample and undetectable with the GeneXpert (>30%) and Hain tests (5-10%).

In addition, although using genetic information from 22 mycobacterial loci only (~13kb total), the Deeplex® Myc-TB assay can detect 97.8% of the mutations associated with MTBC antibiotic resistance detected by whole-genome sequencing (in-silico analysis).

Identification of 156 mycobacterial species

Based on nucleotide identity at the *hsp65* gene, the Deeplex® Myc-TB assay can not only detect M. tuberculosis complex strains but also 156 mycobacterial species, including clinically-relevant species such as *M. ulcerans, M. kansasii* and *M. intracellulare*.

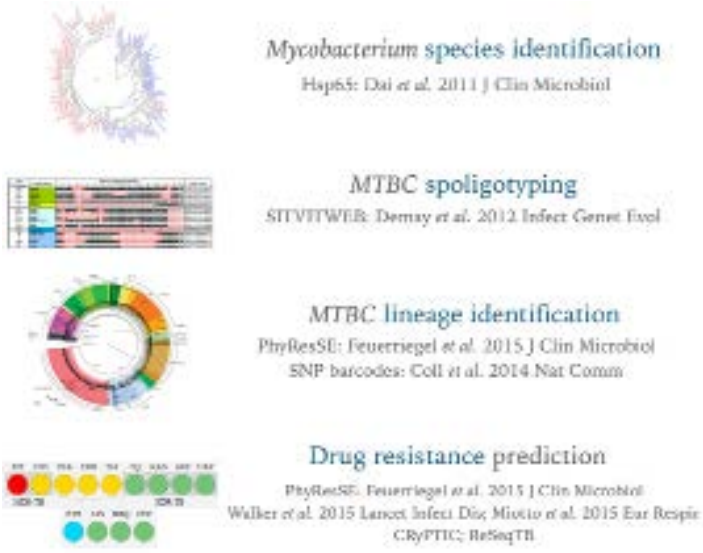


Figure 4. Purposes of the Deeplex® Myc-TB assay.
For each step, **databases** (shown on the right) are interrogated to try and associate observed mutations to mycobacterial species, MTBC lineages or MTBC resistance and susceptibility to antibiotics. Mutations that are not found in the databases are classified as uncharacterized. Spoligotypes are based on the presence-absence pattern at the MTBC CRISPR/DR locus rather than mutations. References for databases, from top to bottom.

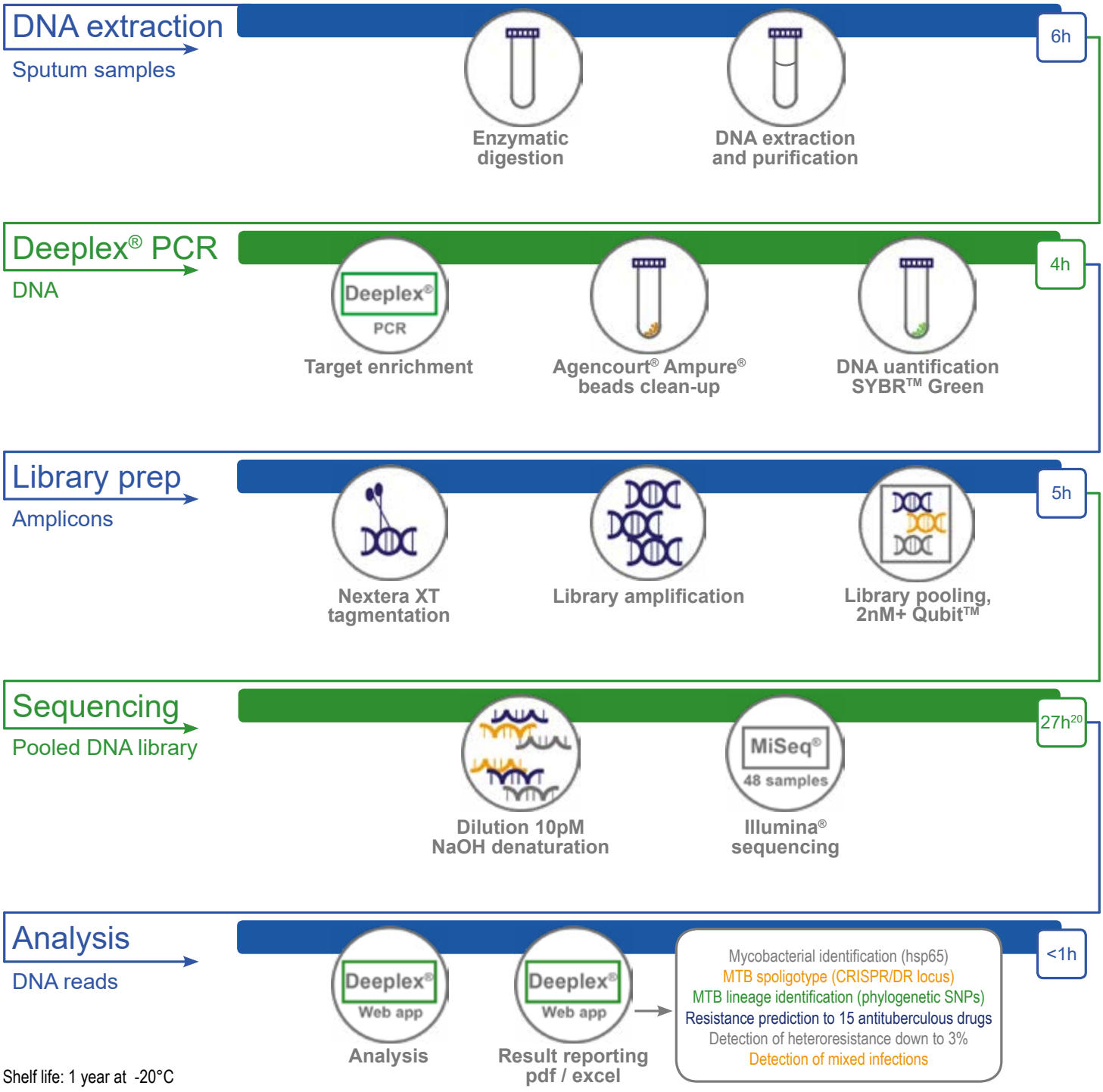
Turn-around time of around 2 days

Mycobacterial cultures are not required for use with the Deeplex® Myc-TB and the assay can be used on complex biological samples. Directly extract DNA, amplify targets with ready-to-use master mix for multiplex PCR, purify the PCR product, prepare sequencing libraries and sequence with the NGS technology of your choice. This should take about two days (Table 1). Once targets are sequenced, output FASTQ (read) files are ready to be uploaded onto our secure web app, using the access code provided with the kit (or by us if using the service). Data will be analyzed with our in-house Deeplex pipeline in less than an hour, results can be visualized directly.

	Deeplex® Myc-TB
Input sample type	gDNA from mycobacterial cultures or from sputa
DNA input quantity	5-10 µl gDNA at 5 ng/µl mycobacterial DNA, 20µl thermolysate, 100µl sputum
Tested library prep	Nextera® XT (Illumina®)
Tested sequencing technologies	Illumina® MiniSeq (21 samples), MiSeq (45), NextSeq (kit: 372; service: 381)*
Turn-around time (kit)	2-3 days
Shipment protocol (service)	water-eluted gDNA or thermo-inactivated† thermolysate
Storage condition	-20°C for up to a year
Kit content	Multiplex PCR master mix, positive (BCG) and internal controls, web app activation code

Table 1. Specifications for use of the Deeplex® Myc-TB kit or service.
Turn-around time includes DNA extraction, multiplex PCR, library preparation, sequencing and analysis. *Number of effective samples – controls not included. †Samples must be inactivated at 95°C for 30 minutes; an inactivation form will be sent to you prior to shipment. Contact us before shipment at contact@genoscreen.fr

Deeplex® Myc-TB workflow



References

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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).
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