DEEPLEX

Deeplex® Myc-TB

From clinical samples to drug resistance profile

A new *Mycobacterium tuberculosis* drug resistance prediction assay,

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

CE IVD

GenoScreen
Innovative Genomics

A fast, deep sequencing-based assay for antibiotic resistance prediction of *Mycobacterium tuberculosis* complex + 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 140 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.

- **Highly sensitive**

  Predict >97%** of MTBC resistance profiles detected by whole genome sequencing, **identify heteroresistance down to 3%** subpopulations and work with DNA loads down to 100 genomes.

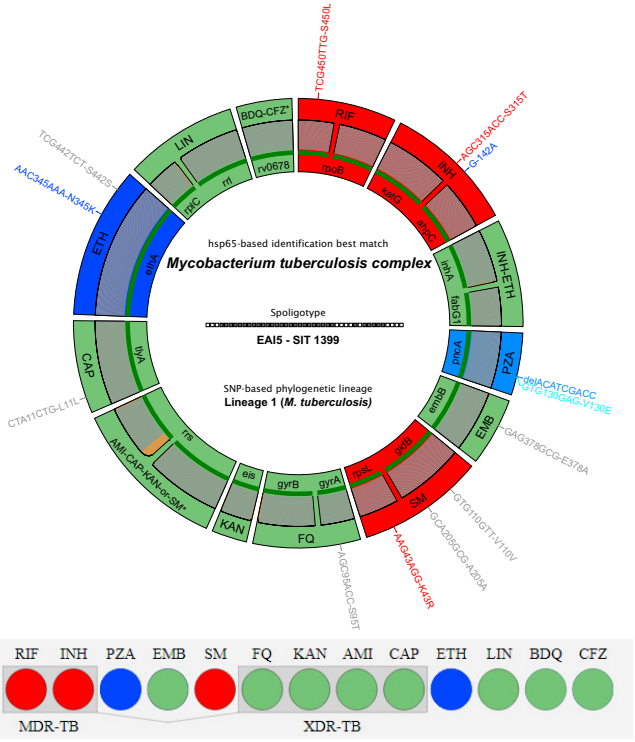


Figure 1. The Deeplex® web app (Top) **Deeplex® map** showing mutations associated (red) or unassociated (or synonym, grey) with antibiotic resistance of MTBC along with yet-to-be characterized mutations (blue). Information on mycobacterial identification is shown in the center of the map. (Bottom) **Resistotype**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

Introduction

According to the World Health Organization, only 186.772 out of the estimated 484.000 incident cases of rifampicin/multidrug resistant tuberculosis in 2018 were detected[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, 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).

An all-in-one 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 assay comes as two options: the service and the kit.

- When using the **Deeplex® Myc-TB** service, GenoScreen performs all steps, from DNA extraction (optional) to final generation of analysed data, made available to the user in the web app.
- Alternatively, users can utilize the **Deeplex® Myc-TB** kit with their samples. The 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.

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

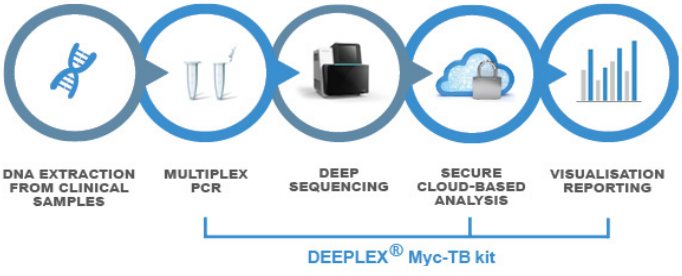


Figure 2. The Deeplex® Myc-TB workflow. From DNA extraction from clinical or culture samples to data analysis and result visualization. The assay comes as two options: the **Deeplex® kit** and the **Deeplex® service**. The kit includes a single PCR master mix ready for multiplex amplification of the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

Prediction of resistance to 15 anti-TB drugs

The **Deeplex® Myc-TB** assay relies on deep sequencing of from 18 main MTBC gene targets associated with resistance to first an 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 including the more recently introduced compounds such as bedaquiline and linezolid[4], making it the most exhaustive genotypic assay directly applicable on specimens available to date.

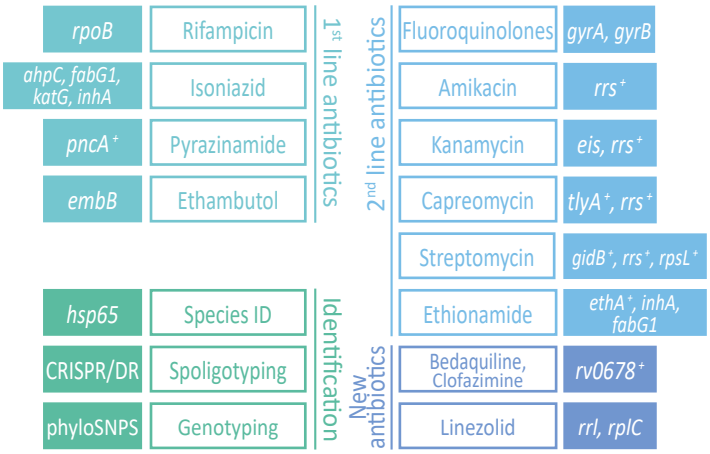


Figure 3. Genes or genes regions amplified and sequenced via the Deeplex® Myc-TB assay (+ : full genes).

MTBC genotyping and spoligotyping

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 3% of bacteria in the sample[5], inaccessible to other rapid molecular tests. Extracted DNA representing as low as 100 mycobacterial genomes, thus below the limit of detection by classical microscopy[5], can be characterized. In addition, although it is targeted, the Deeplex® Myc-TB assay can predict >97% of MTBC resistance phenotypes detected by WGS**.

Identification of more than 140 mycobacterial species

Based on nucleotide identity of the *hsp65* gene, the **Deeplex® Myc-TB** assay can not only indentify *M. tuberculosis* complex but also >140 other mycobacterial species, including most clinically relevant species such as *M. kansasii, M. intracellulare, M. avium* complex...

***Mycobacterium* species identification**	**Hsp65:** Dai *et al.* 2011, J. Clin. Microbiol.
MTBC spoligotyping	**SITVITWEB:** Demay *et al.* 2012, Infect. Genet. Evol.
MTBC lineage identification	**PhyResSE:** Feurriegel *et al.* 2015, J. Clin. Microbiol. **SNP barcodes:** Coll *et al.* 2014 Nat. Comm.
Drug Resistance Prediction	**ReSeq TB** **PhyResSE:** Feurriegel *et al.* 2015 J. Clin. Microbiol. **Data from:** Walker *et al.* 2015 Lancet. Infect. Dis. ; Miotto *et al.* 2015 Eur. Respir.; CRyPTIC

Figure 4. Purposes of the Deeplex® Myc-TB assay.
Upon automated analysis of the sequencing data in the web app, integrated references **databases** (shown on the right) are interrogated to associate observed mutations to mycobacterial species, MTBC lineages, resistance and susceptibility to anti-tuberculosis drugs. Mutations that are unknown to the databases are classified as uncharacterized. Spoligotypes are identified based on the presence-absence pattern of spacers at the MTBC CRISPR/DR locus. References for databases, from top to bottom.

Turnaround time of less than 48 hours

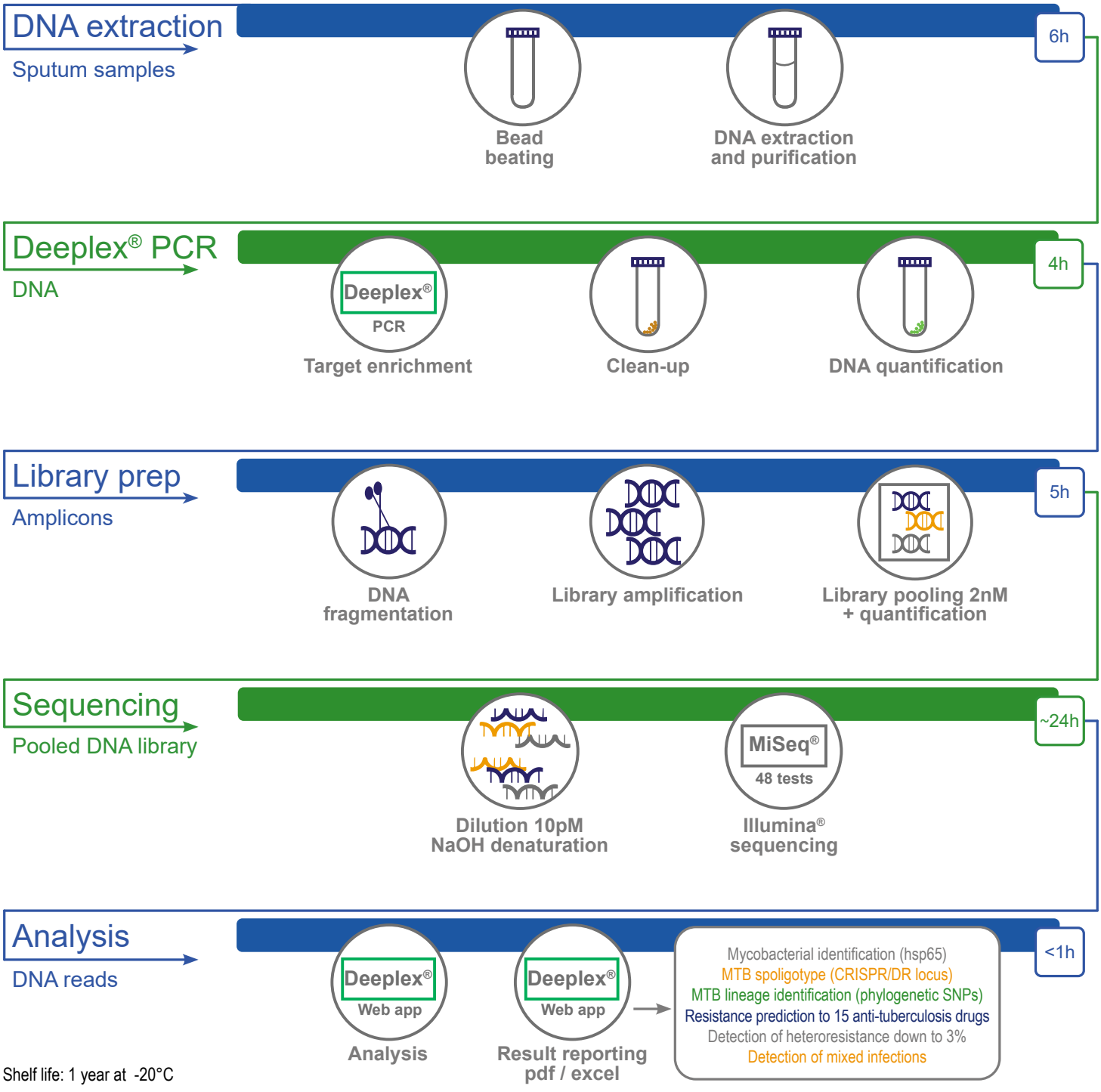
Mycobacterial cultures are not required for use with the **Deeplex® Myc-TB** and the assay can be used on clinical samples with minimal bacterial loads (see above). Directly extract DNA, amplify targets with the ready-to-use master mix for multiplex PCR, purify the PCR product, prepare and sequence DNA libraries. The total takes from 1 to 2 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 fully parameterized Deeplex pipeline in less than an hour, results can be visualized directly

	Deeplex® Myc-TB
Input sample type	gDNA from clinical samples* (eg. sputum...) or culture
DNA input quantity	5-10 µL gDNA at 1 ng/µL mycobacterial DNA, 20 µL culture thermolysate, 200 µL sputum
Tested library prep	Nextera® XT (Illumina®), Nextera® Flex (Illumina®)
Tested sequencing technologies	Illumina® iSeq 100 (13 samples), MiniSeq (21), MiSeq (45), NextSeq (kit: 372; service: 381)[t]
Turnaround time (kit)	iSeq 100: 1 day ; others sequencers: ≈2 days
Shipment protocol (service)	water-eluted gDNA or thermo-inactivated[u] 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.
Turnaround time includes multiplex PCR, library preparation, sequencing and analysis.
[t]Number of effective samples – controls not included.
[u]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

** with genome loads ≥ 100*
*** Results of an in-silico analysis versus the WGS culture-based dataset from «Walker et al, Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study,* Lancet Infect. Dis. ***15****, 1193-1202 (2015)»*

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).
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